Biochimica et Biophysica Acta 1865 (2016) 155–167

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Cellular senescence and tumor promotion: Is aging the key?

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ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 29 January 2016
Accepted 31 January 2016
Available online 2 February 2016

Keywords:
Aging
Cancer
Cellular senescence
Premalignant
Pseudo-senescence
SASP

ABSTRACT

The senescence response is a potent tumor suppressor mechanism characterized by an irreversible growth arrest in response to potentially oncogenic signals to prevent the proliferation of damaged cells. Late in life, some of the features of senescent cells seem to mediate the development of age-related pathologies, including cancer. In the present review, we present a summary of the current knowledge regarding the causes, effector pathways and cellular features of senescence. We also discuss how the senescence response, initially a tumor suppressor mechanism, turns into a tumor promoter apparently as a consequence of aging. We argue that three age-related phenomena—senescence-associated secretory phenotype (SASP) dysregulation, decline in the immune system function and genomic instability—could contribute, independently or synergistically, to deteriorate the efficacy of the senescence response in stopping cancer. As a consequence, senescent cells could be considered premalignant cells, and targeting senescent cells could be a preventive and therapeutic strategy against cancer.

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Abbreviations: SASP, senescence-associated secretory phenotype; DDR, DNA-damage response; OIS, oncogene-induced senescence; ROS, reactive oxygen species; HMGB1, nuclear protein high mobility group box 1; SAHF, senescence-associated heterochromatin foci; SMS, senescence-messaging secretome; NSAID, nonsteroidal anti-inflammatory drugs; SIR, senescence inflammatory response; PML, promyelocytic leukemia protein.

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http://dx.doi.org/10.1016/j.bbcan.2016.01.007
0304-419X/

1. Introduction

Aging represents an inexorable fate and refers to a progressive and generalized deterioration of the functional capacities of an organism. This deterioration results in a high susceptibility to environmental challenges, leading to age-associated pathologies that ultimately cause death [1,2]. However, the fundamental mechanisms that drive the age-related deterioration of cellular and organismal functions are not clearly understood, representing an important obstacle in the development of strategies to promote healthy aging.

Aging is intrinsically a complex process arising from intricate interactions between genetic, environmental and stochastic factors [3]. A key discovery for the understanding of the aging process emerged more than 50 years ago when Hayflick and Moorhead found that human diploid cell strains undergo irreversible growth arrest after extensive serial passages in culture, a phenomenon described as "cellular senescence" [4]. In the following decades, the concept of cellular senescence evolved from being just an in vitro curiosity to being an important biological mechanism involved in tumor suppression [5–7], embryonic development [8–10], wound healing/tissue repair [11,12] and organismal aging [13–15].

Today, senescence is defined as a cellular state characterized by irreversible proliferative arrest that arises as response to diverse stress signals to prevent propagation of damaged cells [16]. Senescence is associated with changes in gene expression and epigenetic profiles that lead to profound phenotypic alterations, including resistance to apoptosis [17,18] and oncogenic transformation, and to the secretion of multiple pro-inflammatory molecules (cytokines, chemokines, growth factors and proteases), globally known as the senescence-associated secretory phenotype (SASP) [13,16,19–22].

The most widely recognized biological function of the senescence response is to prevent cancer development early in life. Indeed, a cell is triggered to permanent growth arrest by oncogenic stimuli, and several onco-suppressive pathways, including the p53-p21-ARF and p16^{INK4a}-pRB, are engaged in developing a fully senescent phenotype [23–26]. However, the senescence response may also be considered a double-edged sword that mediates the development of age-related pathologies. The SASP is an important player in this dual function because pro-inflammatory signals may induce profound alterations in the microenvironment, allowing cancer cells and pathologies to thrive, especially as a function of age [27–30].

This apparent contradiction in the role of cellular senescence in complex organisms could be explained in the evolutionary context as a consequence of antagonistic pleiotropy [20]. The evolutionary theory of aging predicts that processes that ensure fitness early in life will be selected even if they lead to deleterious effects at late stages, such as aging phenotypes [2,19,32]. This occurs because natural mortality is mainly caused by extrinsic hazards, such as infection, predation, starvation or cold, thus preventing most of the individuals in a population from reaching advanced ages [2]. For cellular senescence, it has been hypothesized that its capacity to suppress the proliferation of potentially dangerous cells early in life can contribute to organismal aging by depleting tissues from functional cells required to maintain homeostasis [14,19–21].

Senescent cells accumulate in different tissues in aging mammals [14,33]. However, the causes and consequences of this age-associated accumulation are largely unknown. Interventions to clear senescent cells from a progeroid mouse model seem to have a positive impact by delaying the onset of aging phenotypes and attenuating the progression of already established disorders [34], suggesting that senescent cells contribute to develop the aging phenotypes. Moreover, a recent study suggests that age-related changes [35] in the ability of cells to cope with stressing stimuli can be responsible for an inappropriate senescence response, consequently leading to a pathology [35].

It is not yet possible to assess whether the detrimental phenotypes associated with the senescence response at advanced ages are a cause or a consequence of aging. Aging leads to changes in gene expression and chromatin structure that could be responsible for the decline in cellular and physiological functions [36], and the senescence response may as well be part of the overall aging effect. Further complications arise on how the senescence response can be implicated in cancer development. Different reports demonstrated that interfering with specific phenotypes of senescent cells can be a valuable intervention in slowing cancer initiation and progression [37,38]. Therefore, future research might be able to provide insights into the design of novel strategies to prevent and treat cancer, and possibly other age-related diseases.

In the present review, we summarize the current knowledge about the causes, effector pathways, cellular features and consequences of senescence. We explore how the senescence response, initially a tumor suppressor mechanism, becomes a tumor promoter as a consequence of aging. We discuss how three age-related phenomena—decline in the immune system function, genomic instability and SASP dysregulation—could progressively deteriorate the senescence response, thus contributing to cancer development. Consequently, the potential premalignant properties of senescent cells might serve as targets for preventive and therapeutic strategy against cancer.

2. Triggers and effector pathways of senescence

Senescence can be induced by different stimuli, including telomere attrition, DNA damage, chromatin perturbations and oncogene activation. These triggers initiate signaling cascades that, dependently or independently of DNA-damage response (DDR), activate p53–p21 and/or p16^{INK4a}–pRB tumor suppressor pathways, which promote proliferation arrest and senescence response (Fig. 1).

2.1. Telomere attrition

The limited replicative potential of human cells, initially described in vitro by Hayflick and colleagues [4], is well-understood today as a consequence of telomere shortening. Due to the intrinsic mechanism of DNA replication, successive divisions cause progressive decrease in telomeres length, which eventually become critically short and dysfunctional [39]. This telomere attrition activates a DNA damage response without effective repair [40], leading to cell cycle arrest mediated by p53 and to the induction of the senescent phenotype [41] (Fig. 1).

2.2. DNA damage

Strong genotoxic stress such as ionizing radiation, topoisomerase inhibitors and oxidative agents can cause DNA double-strand breaks (DSB) across the genome [42], which induce chronic DDR signaling, p53 activation and cellular senescence [43] (Fig. 1). The activation of ATR and ATM, two of the main components of DDR signaling, is a key event in the senescence cascade leading to growth arrest [44–46]. Interestingly, ATM activation by deacetylase inhibitor, Trichostatin A, can also initiate DDR in the absence of actual DNA breaks [45].

2.3. Oncogene activation

Consistent with its role as a tumor suppressor mechanism, senescence is also induced upon strong mitogenic signals. The first example of oncogene-induced senescence (OIS) was described by Serrano et al., who found that expression of oncogenic *Ras* in primary human and rodent cells resulted in a permanent G1 arrest, mediated by p53 and p16^{INK4a}, and in the induction of a phenotype indistinguishable from replicative senescence [7]. In this case, the cell cycle arrest through DDR signaling is triggered by the production of reactive oxygen species (ROS) [47–50] and aberrant DNA replication, which lead to persistent DSB [51,52]. Currently, several oncogenes are known to promote OIS, including Raf, Akt, cyclin E and others [24,25,51,53]. The aberrant expression of oncogenes is not the only mitogenic signal that can induce

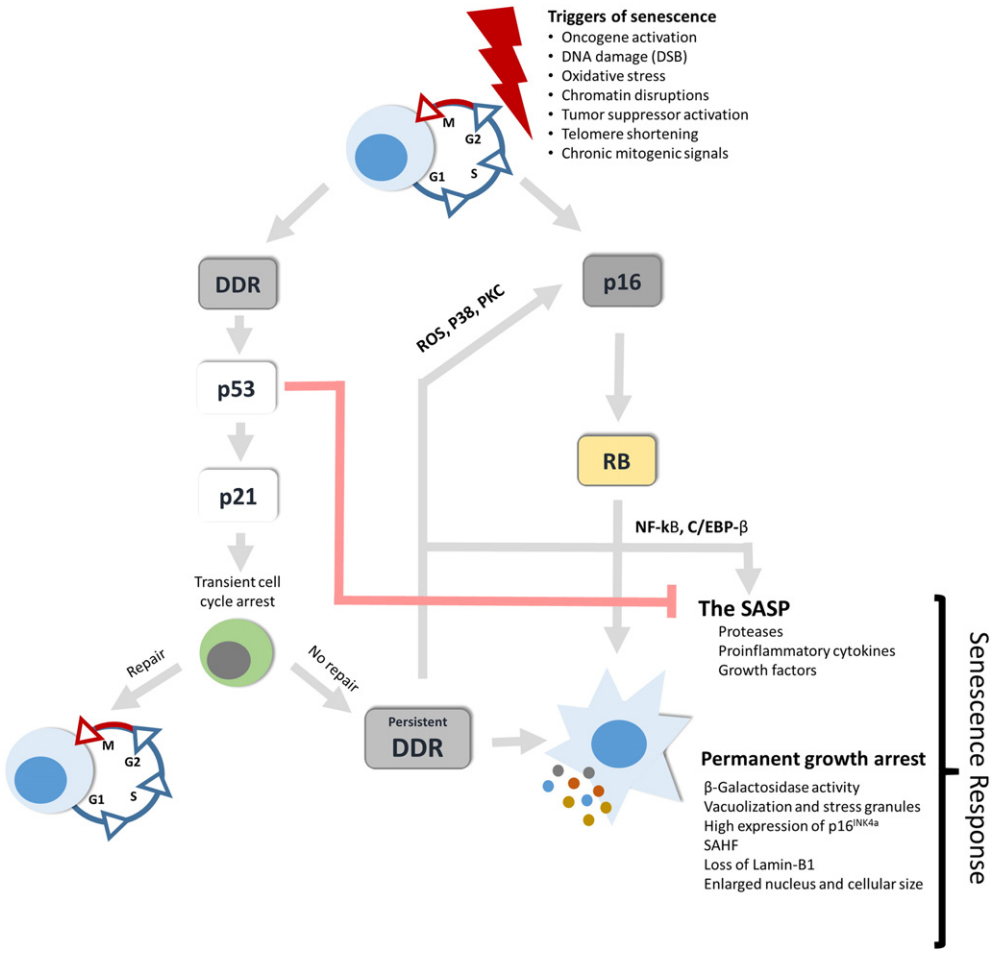

Fig. 1. Senescence response: triggers, pathways, features and markers. Diverse stressing stimuli initiate DNA damage response (DDR), which initially induce a transient p53/p21-dependent cell cycle arrest, to allow time for repair. If this does not occur, persistent DDR signaling leads to permanent proliferative arrest by engaging the $p16^{INK4a}$–pRB pathway. $P16^{INK4a}$ is activated by p38MAPK, PKC and reactive oxygen species (ROS) signaling; senescence-associated secretory phenotype (SASP) is negatively regulated by p53 and positively regulated by NF-κB and C/EBP-β. SAHF = senescence-associated heterochromatin foci.

senescence. Indeed, prolonged exposure to interferon-β triggers a senescence program that involves p53 activation and DNA damage signaling, which is activated by the accumulation of ROS [54].

2.4. Epigenetic perturbations

Changes in chromatin organization also trigger the senescence response [19], although the mechanisms are not well understood. One possibility is that the disruption of heterochromatin by chromatin relaxation may lead to a senescent state. This has been shown to occur through a p53- and telomere-independent mechanisms partly mediated by histone deacetylase agents, which promote de-repression of the $p16^{INK4a}$ tumor suppressor [55]. Another possibility relies on indirect DDR signaling activation: as mentioned before, deacetylase inhibitors, which induce senescence, can activate ATM and induce DDR signaling without actual DNA damage [44,56].

2.5. Tumor suppressor pathways

Chronic activation of tumor suppressors is a fundamental step in the induction and maintenance of the senescence state, with p53–p21 and $p16^{INK4a}$–pRB being the two main tumor suppressor pathways responsible for the replicative arrest of senescent cells [7,57,58]. These pathways are complex and cross-regulate each other [59] but can also independently lead to senescence (Fig. 1). In general terms, p53 induction of p21 is responsible for the initial and transient cell-cycle arrest induced by DDR signaling, and subsequent activation of $p16^{INK4a}$ and pRB ensures the irreversibility of the arrest [60,61]. Interestingly, DDR signaling pathways seem not to affect the activation of $p16^{INK4a}$, which can be upregulated independently of telomere shortening or DNA damage, indicating that distinct senescence programs can progress in parallel, with different subsets of cells responding to multiple signals [41]. Instead, $p16^{INK4a}$ is responsive to more diverse stresses through distinct regulatory pathways, including Polycomb group complexes (PcGs) [62,63] and stress-activated p38-MAP kinases [62, 64–66]. Even though it is not clearly understood, senescence can also be induced independently of p53 and $p16^{INK4a}$. For example, expression of $BRAF^{V600E}$ in nevi induces a senescence response where factors other than $p16^{INK4a}$ contribute to the protection against malignant transformation [25]. Additionally, Raf-1-induced growth arrest in human mammary epithelial cells occurs independently of $p16^{INK4a}$ expression levels or p53 and pRB inactivation, but the underlying mechanism is still unknown [67].

3. Features and markers of senescent cells

In addition to growth arrest, senescent cells exhibit several features that collectively characterize the senescence phenotype. None of these changes are exclusive, making the identification of senescent cells challenging, especially in vivo, and requiring the use of combinations of markers and characteristics (Fig. 1) [16].

3.1. Morphological and enzymatic changes

Senescent cells are metabolically active and exhibit enlarged cellular size, reflecting the continuation of macromolecule synthesis without cell division. Other morphological changes include flattening, vacuolization, and accumulation of stress granules response [19]. The overexpression of the acidic B-galactosidase is a common marker to detect senescent cells in culture and in tissues [33]. It results from increased lysosomal activity, but it is not necessary to induce senescence [68]. The nuclear morphology is altered during cellular senescence, and correlates with changes in gene expression, particularly with gene silencing [69]. Senescent cells display larger nuclei, irregular nuclear envelope, changes in the composition of the nuclear lamina and in chromosome condensation and distribution [70–72]. Another feature of senescent cells is the relocalization of the nuclear protein high-mobility group box 1 (HMGB1) to the extracellular space, which stimulates cytokine production through TLR-4 signaling [73].

3.2. Chromatin reorganization

As mentioned before, the signaling triggered by DNA damage is fundamental for the induction of senescence. At dysfunctional telomeres and at non-telomeric sites, senescent cells present DNA damage foci, such as γH2AX or 53BP1, which activate the (ATM)–p53–p21 signaling required for growth arrest [51,52,74]. However, these foci do not constitute a specific marker because most cells can repair the DNA damage without undergoing senescence, and because the DDR signaling can be activated without actual DNA damage, as it occurs upon treatment with histone deacetylase inhibitors [16,44,55]. Another chromatin signature in senescent cells is represented by the senescence-associated heterochromatin foci (SAHF), which are chromatin domains stained densely by DAPI and rich in H3K9me3 and HP1, possibly at silenced pro-proliferative genes [75]. However, SAHF are detected only in vitro and they have not been observed in tissues positive for $p16^{INK4a}$ expression and possessing other features of cellular senescence [76]. The loss of lamin B1 is also observed in senescent cells, which has been found to be a key factor in chromatin reorganization during senescence, including dramatic changes in the distribution of trimethylation on histone H3 (H3K4me3 and H3K27me3), specifically the formation of large-scale domains rich in H3K4me3 and H3K27me3 ("mesas") and others depleted of H3K27me3 ("canyons") [77].

3.3. Gene and protein expression changes

Gene expression profile during senescence is profoundly affected. Increased expression of $p16^{INK4a}$ is a common marker to identify senescent cells in vivo and in vitro [78,19,16]. This protein is generally absent in healthy young cells, but it becomes progressively upregulated with aging and can also be detected in other types of senescent cells [20, 79]. For example, it can be transiently upregulated during optimal wound healing [11,80]. Other important changes in gene expression, already mentioned before, correspond to the loss of lamin B1 [70,77] and the activation of the p53 pathway [7,57,58].

The profound changes in gene expression taking place during senescence are also reflected in the remarkable variety of molecules secreted by senescent cells, including enzymes that degrade the extracellular matrix, immune modulators, inflammatory cytokines, growth factors and others [13,19,22]. Collectively, this is called SASP and has been associated with tumor suppression, wound healing, immune modulation and evasion as well as with tissue remodeling and age-related pathologies.

4. The SASP

The induction of senescence is accompanied by a complex pro-inflammatory response denominated SASP or senescence-messaging secretome (SMS), composed of pro-inflammatory cytokines (e.g. IL-1α, IL-1β, IL-6 and IL-8), growth factors (e.g. HGF, TGFβ and GM-CSF), chemokines (e.g. CXCL-1, -3 and -10) and matrix remodeling enzymes (e.g. MMPs) [22,29,81]. The diverse biochemical activities induced by the components of the SASP suggest that it constitutes a mechanism to communicate with other cells and to modulate the local microenvironment.

4.1. Regulation

Cells that undergo senescence due to ectopic overexpression of p21 or $p16^{INK4a}$ experience growth arrest and display several features of the senescent phenotype but do not develop a SASP [82]. The SASP develops slowly over several days [83], mostly as a consequence persistent DNA damage signaling, and it is positively regulated by several proteins acting upstream in the DDR cascade such as ATM, NBS1 and CHK2 (Fig. 1) [29,84]. Surprisingly, p53 has an inhibitory effect, and its inactivation in senescent cells causes hyper expression of SASP [29]. The SASP is also regulated by NF-κB [27,56,60] and C/EBP-β, which are transcription factors that modulate immune and inflammatory responses. Recently, the transcription factor GATA4 has been described as a novel senescence regulator, required for the induction of the SASP. After damaging stimuli, GATA4 is stabilized to activate the transcription factor NF-κB, which initiates the SASP and facilitates progression to senescence. In accordance with previous studies, GATA4 is activated by the DNA damage response regulators ATM and ATR, but not by p53 or $p16^{INK4a}$ [85].

4.2. Biological functions

Some components of the SASP help the tumor-suppressive function of the senescence response. For example, IL-6, IL-8, IGFBP7, GROα and WNT16B reinforce the senescence state after the activation of oncogenes and prevent malignant transformation [27,86–89]. However, other reports have found that the factors of the SASP have potent protumorigenic properties. Senescent human fibroblasts can stimulate premalignant and malignant epithelial cells to proliferate in vitro and form tumors in vivo. This effect was promoted, at least in part, by soluble and insoluble factors secreted by senescent cells after exposure to stressing stimuli such as replicative exhaustion, oncogenic Ras, $p14^{ARF}$ or hydrogen peroxide [30]. Additionally, the SASP induced by genotoxic stress favors the emergence, maintenance and migration of cancer stem-like cells [90], supporting a tumor promoting effect by the SASP (Fig. 2).

It has been demonstrated in vivo and in vitro that the SASP of OIS cells can induce paracrine senescence in normal cells, reinforcing and spreading senescence to neighboring cells and tissues. This paracrine effect is mediated by the inflammasome (e.g., TGF-β family ligands, VEGF, CCL2 and CCL20) and IL-1 signaling [91] and could promote immune clearance [92]. Whether this effect is beneficial or detrimental is unknown and probably highly dependent on the specific cellular context. Moreover, this paracrine induction of senescence could also mediate $p16^{INK4a}$ expression in the surrounding stromal and infiltrated immune cells reported by Burd and colleagues [93]. In this case, the tumor could be inducing paracrine senescence through a SASP-like secretion that includes factors able to induce senescence. However, more studies are necessary to unravel the role of paracrine senescence in disease progression before it can be considered for therapeutic applications targeting cancer cells.

Although several SASP factors are context dependent, there is a substantial overlap across different cell types, including normal and tumor cells. For example, IL-6 and IL-8 are among the most conserved factors and can stimulate angiogenesis, disrupt cell–cell communication, impede macrophage function, induce innate immune responses, and promote epithelial and endothelial cell migration and invasion (Fig. 2) [32, 94–96]. All these events help to explain the pro-tumorigenic effects of the SASP. In premalignant cells, loss of p53 or gain of oncogenic Ras exacerbated the tumorigenic effect of the SASP, including epithelial–

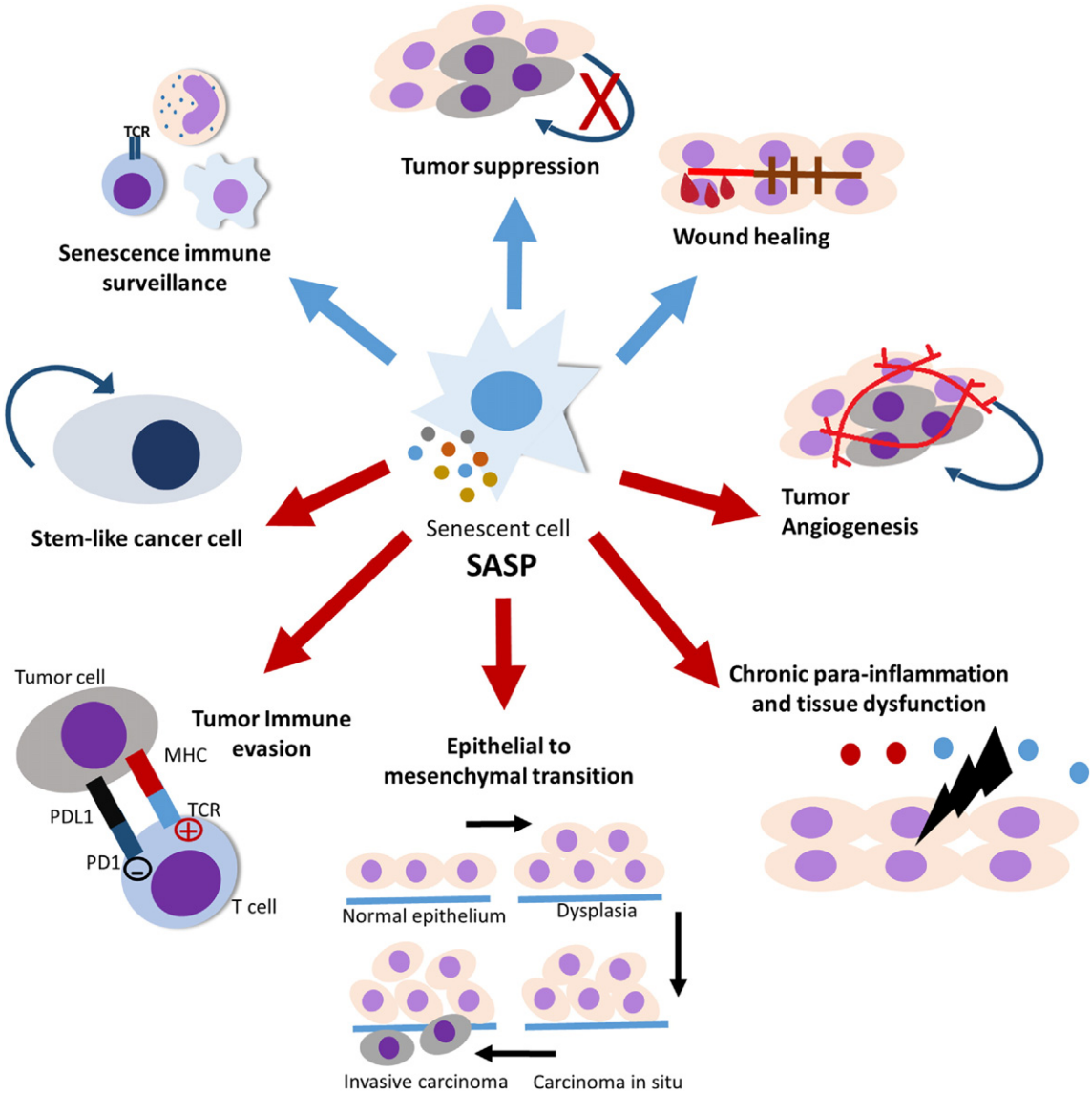

Fig. 2. Opposite functions of the SASP. The senescence-associated secretory phenotype (SASP) has been shown to cause diverse effects in senescent cells and their neighbor cells. Some of these effects are beneficial, such as the activation of the immune system, promotion of wound healing and tissue repair and suppression of tumors (blue arrows). However, detrimental effects, including the promotion of tumor immune evasion and angiogenesis, stem cell-like phenotype in malignant cells, chronic inflammation and epithelial to mesenchymal transition and metastasis, have been reported (red arrows).

mesenchyme transition and invasiveness (Fig. 2), which seemed to be mediated by a paracrine effect of IL-6 and IL-8 [29].

5. Aging and the senescence response

Current evidence indicates that cellular senescence functions as a potent tumor suppressor mechanism that acts in coordination with the immune system to clear potentially malignant cells from the tissues: upon oncogenic or damaging stimuli, the senescence response induces proliferative arrest to prevent propagation of the damaged cells. The induction of SASP attracts the immune system and promotes the clearance of senescent cells as well as tissue repair.

However, besides this tumor suppressor capacity, the senescence response also seems to be involved in tumorigenesis. How can the same mechanism promote two opposite events? One possible explanation is that gene expression and epigenetic alterations that accompany aging alter the normal senescence response, thus interfering with its anti-tumorigenic activity and increasing the risk of cancer. It can be hypothesized that age-related deterioration of biological functions might lead to cellular and organismal changes that alter the senescence response and contribute to transforming it into a tumor-promoting mechanism (Fig. 3).

Supporting this hypothesis, a recent study reported that age is a critical factor in mouse senescence response against oncogene activation. Golomb and colleagues showed that mutant H-Ras activation in mouse epidermis induced a differential outcome determined by age [35]. Young skin responded with hyperplasia while old skin developed dysplasia and gradual progression towards carcinoma. This impaired response in old mice was characterized by exacerbated inflammation and accumulation of immune cells as well as excessive cellular senescence. The inflammatory response showed age-dependent increase of pro-inflammatory cytokines, but also activation of a strong anti-inflammatory Th2 response. Moreover, the expression of Pdl1, a ligand that promotes cancer immune evasion [97], was upregulated in the aged mice [35] (Figs. 2 and 3).

The accumulation of senescent cells seems to be detrimental for mammals, since it appears to mediate aging features and promote tumorigenesis. However, the cause of their accumulation and how it contributes to tumorigenesis is not completely understood. Different features associated with age might contribute, independently or

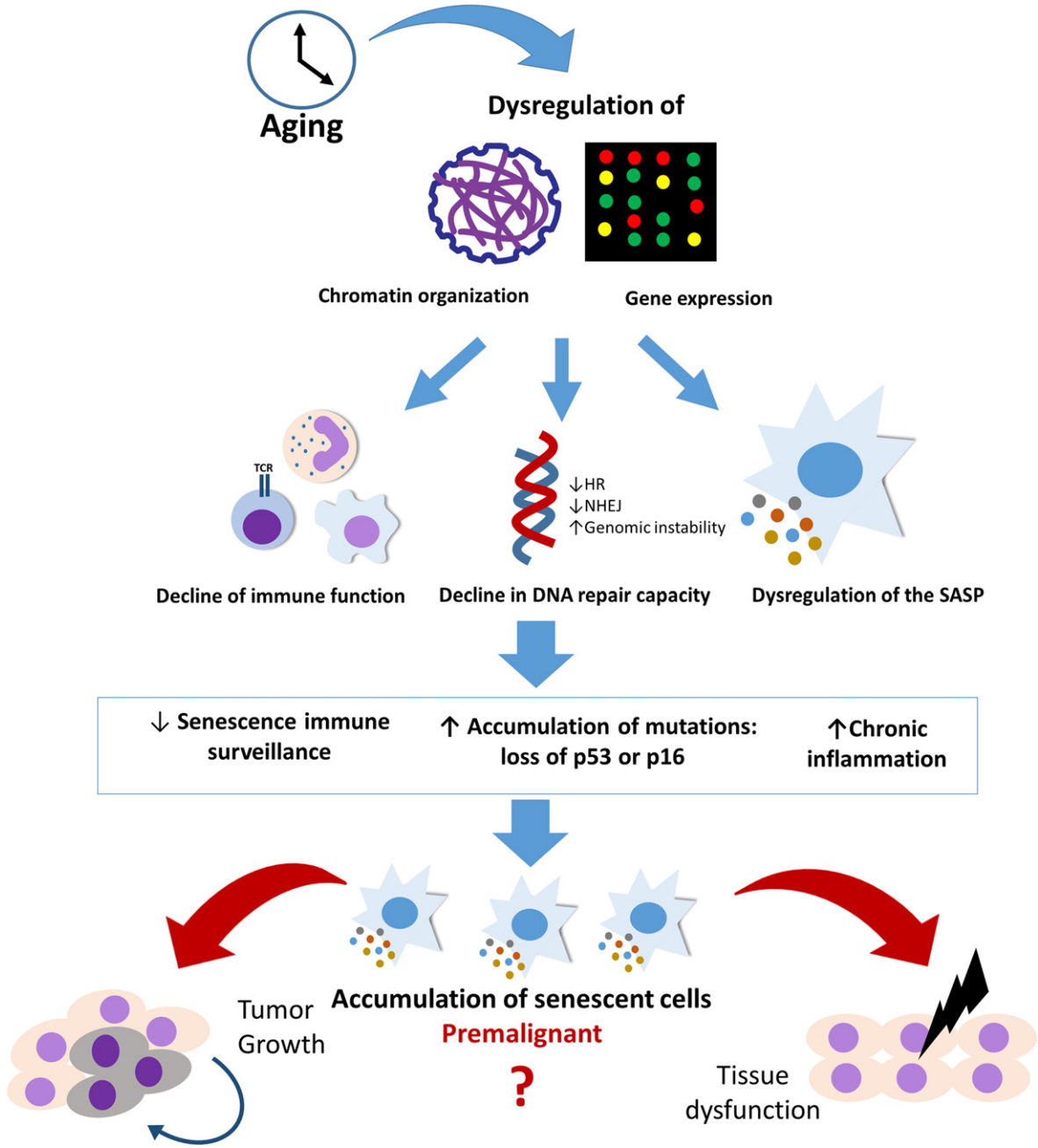

Fig. 3. Age-associated changes in chromatin organization and gene expression. Changes in chromatin and gene expression are responsible for the deterioration of multiple cellular functions, including the senescence response. The decline in the immune system and DNA repair capacity together with a dysregulated senescence-associated secretory phenotype (SASP) in aged cells could contribute synergistically to the accumulation of senescent cells, a process that is associated with tumor development and age-related pathologies. HR = homologous recombination; NHEJ = non-homologous end joining.

synergistically, to promote tumorigenesis in the senescent cells and/or their surrounding non-senescent cells: SASP dysregulation, deterioration of the immune system and impaired DNA repair activity (Fig. 3).

5.1. SASP dysregulation

As discussed above, the SASP presents variations depending on tissue and stimulus. However, inflammatory cytokines such as IL-6 and IL-8 are highly conserved and have a major role in maintaining the SASP response in senescent cells and the surrounding tissue [22].

The functions of the SASP are diverse and intriguing as different studies show opposing and contradictory effects. Through the SASP, cells communicate their compromised states to neighbor cells, to promote tissue repair, reinforce senescence and activate immune surveillance. Paradoxically, SASP also seems to have pro-tumorigenic properties promoted by chronic inflammation [91]. The inflammatory cytokines in the SASP are thought to drive aging and age-related disease [83], including cancer (Fig. 2).

A study by Pribluda and colleagues described, in a mouse model of colorectal cancer, a novel senescence-related inflammatory phenotype, defined as the senescence inflammatory response (SIR). The SIR was characterized by a low-grade atypical inflammatory response stimulated upon oncogene-induced senescence in epithelial cells. It included predominantly upregulation of genes related to innate immunity and lack of cellular inflammatory infiltrate in the senescent tissues. Furthermore, the SIR was found to either repress or promote tumorigenesis in a p53-dependent fashion, as growth and invasiveness were stimulated by

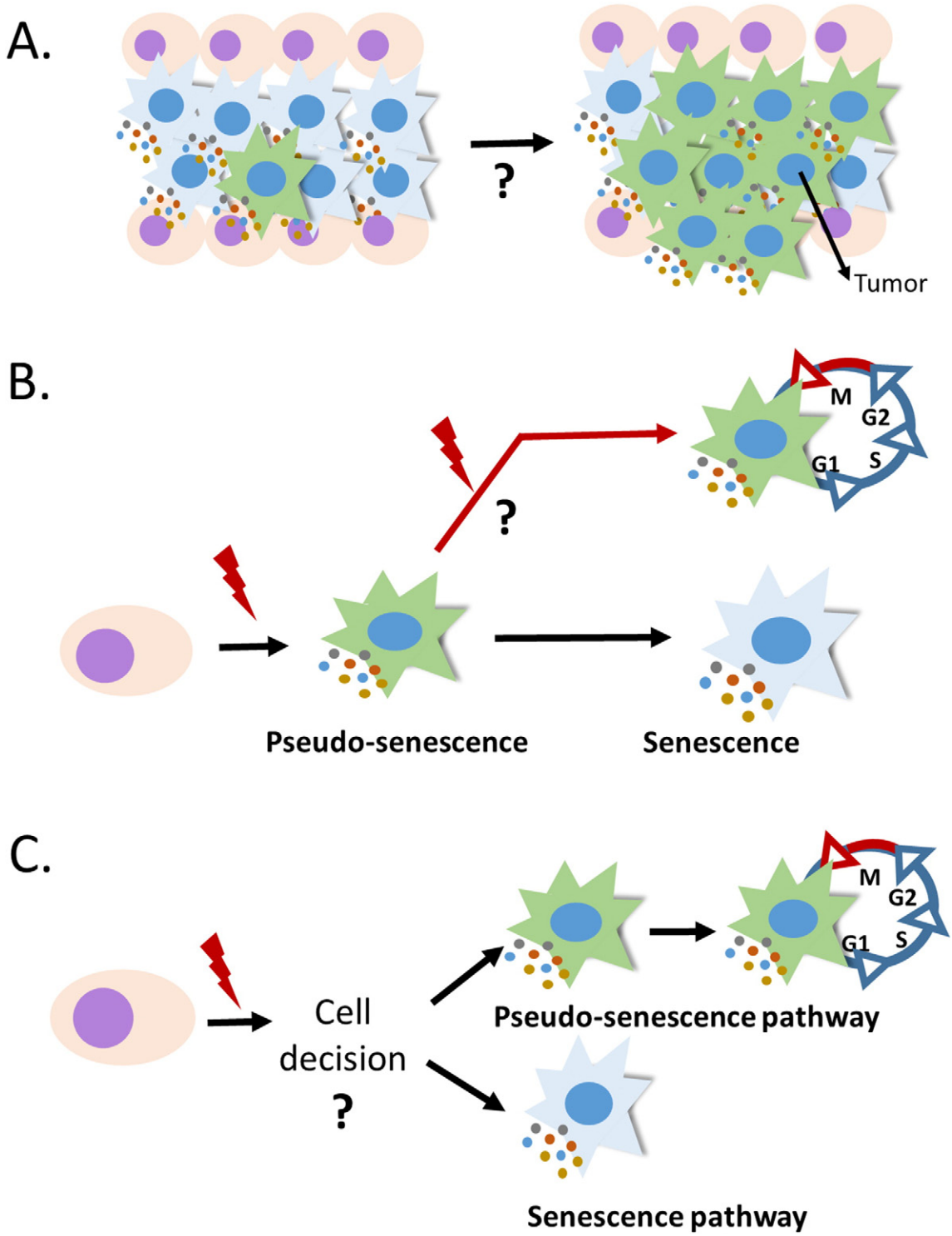

Fig. 4. "Bypass" of the senescent arrest. (A) Rare clone. A rare clone masked within a population of senescent cells is responsible for the progression to cancer. This clone may share some of the typical markers of senescent cells, but it retains the capacity to proliferate. (B) Pseudo-senescence as an intermediate state before reaching fully irreversible senescence. Cells in this state could express most of the senescent makers but retain the capacity to resume proliferation if additional stimuli deviate their normal progression towards the terminal senescent state. (C) Pseudo-senescence response as an alternative response to stress. Cells in this state could express most of their senescence markers but still retain the capacity to resume proliferation. This state emerges as an alternative aberrant response against an oncogenic stimulus, which could become more prevalent with aging.

the absence of p53 [37]. This observation is in line with other studies reporting that the inactivation of p53 in senescent cells induces hyper expression of the SASP [29,84]. The SIR seems to be mostly a cell-autonomous process that presents little overlap with the previously described SASP, suggesting that the two phenotypes are regulated by different transcription factors and probably have distinct physiological roles [37].

The intrinsic characteristics of the SIR correspond to the definition of para-inflammation [22]: low-grade inflammation associated with many chronic diseases including diabetes and neurodegeneration [98].

Interestingly, the work by Pribluda et al. also showed the important role of inflammation in tumor progression as the suppression of the SIR after nonsteroidal anti-inflammatory drugs (NSAID) treatment prevented carcinogenesis [37]. It is unknown whether or not the SIR is induced in all senescent cells or at specific stages during the progression to senescence, but it is very likely that this might be a phenotype highly specific for the type of tissue and stress involved. One possibility is that the SIR may represent the beginning phase of the inflammatory process, which will eventually evolve into a full SASP [22].

The SASP also includes proteins that can promote immune evasion. Senescent cells can secrete high levels of matrix metalloproteinases (MMPs) [99], which have been recently associated with poor anti-tumor immune response in melanoma patients [100] and seem to be upregulated during aging [101], suggesting a connection between aging, senescence and immune evasion. Furthermore, the programmed death-ligand 1 (PDL-1), which can suppress immune recognition and elimination of numerous types of cancers [102], has been reported to be upregulated in the skin of aged mice and in the context of OIS [35], supporting the promoting effect of immune evasion by aged senescent cells.

NF-κB is also known to be involved in aging and in senescence, and could constitute a link to the altered senescence response observed at advanced age. In a transgenic mouse model subject to conditional inhibition of NF-κB in the skin, the normal aging phenotype in the skin showed rapid reversion to a more youthful state after NF-κB inhibition and reduction in the expression of $p16^{INK4a}$ [103], suggesting that increased NF-κB activity is necessary to maintain the aged phenotype [104]. Moreover, NF-κB is stochastically activated in a variety of cell types in aged mice and its inhibition reduces oxidative DNA damage and delayed cellular senescence in wild-type and progeroid mice [105], although the exact mechanisms underlying this observations are still unknown. Furthermore, it has been found that NF-κB functions as a master regulator of the SASP in OIS by influencing the expression of multiple genes. In cultured fibroblasts, NF-κB suppression also promoted the escape from immune recognition by natural killer cells and cooperated with p53 inactivation to bypass senescence [106].

Another recent study reported a connection between the SASP, NF-κB and mTOR (target of rapamycin) [107], a well-known pathway involved in aging and tumorigenesis [108]. Laberge and colleagues reported that mTOR inhibition with rapamycin decreased the pro-inflammatory phenotype of senescent cells. Specifically, it selectively suppressed translation of the membrane-bound cytokine IL1A, diminishing NF-κB transcriptional activity, affecting the SASP and reducing the ability of senescent fibroblasts to induce prostate tumor growth in mice. Based on these observations, the authors concluded that rapamycin is a good drug candidate to prevent age-related pathologies, including cancer, due to its capacity to reduce senescence-associated inflammation [107].

NF-κB is critically involved in regulating the immune system and could reduce the efficiency of senescent cells in attracting the immune system and promoting tissue repair. Based on current evidence, it can be hypothesized that during aging chronic NF-κB activation in senescent cells, combined with age-related decline in the immune system, leads to inefficient clearing of senescent cells and exacerbation of the aging phenotype, contributing to increased risk of cancer [22]. Combining mice models of NF-κB activation, senescence and aging can provide important insights into the mechanisms used by this transcription factor in modulating senescence response during aging, in altering SASP and the immune system, thus leading to cancer development.

5.2. Deterioration of the immune system

Tumor suppression by the senescent response involves cooperative interactions with the immune system. This was initially described in a mouse model of p53-deficient liver carcinoma where a brief reactivation of endogenous p53 could induce complete tumor regression through the activation of a senescence response and of the innate immune system, responsible to target and clear the tumor cells in vivo [109].

More recently, the adaptive immune system was also confirmed as a key element in the tumor barrier mediated by senescence. In a study by Kang and colleagues [38], oncogenic expression of N-Ras triggered senescence in hepatocytes in vivo and secretion of chemokines and cytokines that attacked immune cells, including CD4+ T-cells and macrophages, which mediated the clearance of senescent cells. Furthermore, impairments in the immune system resulted in the development of murine hepatocellular carcinomas, showing that senescence surveillance is important for tumor suppression in vivo. Interestingly, in the mice expressing N-Ras, Th1 lymphocytes recognizing the mutated region of the $Nras^{G12V}$ protein were detected. This reveals a remarkable specificity of the response and a primordial role for the adaptive immune system in senescent cell clearance and suppression of liver cancer, in contrast with previous findings describing a major role for the innate immune system in senescent cell clearance [109]. Immune clearance of senescent cells seems to occur also in humans; indeed, immunocompromised patients, under immunosuppressive therapy or with an HIV infection, presented accumulation of senescent cells in the liver [38].

The importance of the cooperation between senescent cells and the immune system has also been shown for tissue regeneration. In a liver fibrosis murine model, it was observed that natural killer cells preferentially kill senescent stellate cells in vitro and in vivo, thereby facilitating the resolution of fibrosis after acute tissue damage [110]. This finding indicates that efficient tissue repair requires activation of the immune system. Interestingly, these two functions are known to be altered during aging, suggesting that the reduced capacity for tissue repair observed during aging could be, at least in part, a consequence of the age-related deterioration of the immune system.

The progressive decline in tissue regenerative capacities during aging has been attributed mainly to degenerative changes in tissue-specific stem cells as well as in their niches and the systemic signals that regulate stem cell activity [111]. However, it can also be connected with the decline of immune system function and the accumulation of senescent cells. Indeed, ineffective immune clearance of senescent cells can generate a persistent microenvironment that negatively affects the regenerative capacity of stem cells. It is essential to understand the link between aging, tissue repair, senescence, the immune system and tumorigenesis since tumors have been described as “wounds that don't heal” [112,113] due to the numerous similarities between cancer and wound healing.

Senescence surveillance seems to be an important extrinsic component in the tumor suppression mechanism imposed. However, current evidence on immune-mediated clearance of senescent cells comes from studies focusing specifically on the liver, opening the question of whether this process also occurs in other organs. Melanocytic nevi, clonal and benign tumors of cutaneous melanocytes, exhibit accumulation of senescent cells without immune response [25,114], suggesting that immune clearance of senescent cells is regulated in complex ways and might be more efficient in certain tissues than others, possibly due to differential expression of SASP components in specific cells. However, nevi typically remain in a growth-arrested state for decades and only rarely progress into malignancy [25], indicating that the immune system might not always be necessary to prevent cancer. Studying the differences of senescence and immune responses between skin and liver seems to be a promising starting point to identify factors that are secreted or expressed in senescent cells and to determine immune recognition and clearance.

Furthermore, investigation of the link between age-related deterioration of the immune system and the senescence response could provide important insights into their relative contribution to cancer progression. This may require sophisticated in vivo modeling, but recent advances such as the *INK-ATTAC*, *p16-3MR* or $p16^{LUC}$ mouse models [11, 34,93] and others could facilitate to answer these complex questions.

5.3. Impaired DNA repair

Age-related decline in cellular capacity to repair the DNA has been extensively studied in vivo and in vitro [115]. Old cells express lower levels of DNA repair proteins and show lower efficiency and higher rate of errors. Homologous recombination (HR), a highly accurate mechanism for DSB repair dependent on cell cycle [116], declines sharply with increasing replicative age in normal human fibroblast, showing up to 38-fold decrease in HR efficiency when comparing pre-senescent and young cells [117]. HR is a repair mechanism dependent on cell cycle; therefore, senescent cells have an intrinsic limited DNA repair capacity as their cell cycle arrest prevents DSB repair through HR, allowing repair only through non-homologous end joining (NHEJ) pathway, which is a DSB repair mechanism more prone to error than HR, but independent of cell cycle [118]. Remarkably, NHEJ pathway seems to be also altered during senescence. A study in senescent normal human fibroblasts showed a 4.5-fold decrease in NHEJ efficiency in pre-senescent and senescent cells compared with young cells, and the frequency of precise ligation was higher in young cells, whereas in old cells extended deletions were more frequently observed [119].

According to this evidence, senescence might represent an intrinsically genomic unstable state, highly prone to accumulate additional genetic mutations, thus constituting a fertile niche for the progress of malignant transformation (Fig. 3). However, all these studies were carried out in models of replicative senescence and provide little insight into the DNA repair capacity status when senescence is induced by different types of stress. Whether the same decline in DNA repair is observed under other types of senescent-inducing stimuli and whether this phenomenon can occur in vivo have still to be addressed. As replicative telomere shortening induces chronic activation of the DNA repair machinery leading to its exhaustion, it could be possible that other types of senescence do not present the same alterations in DNA repair capacity, especially the senescence responses induced through DDR-independent pathways.

Genomic instability is not sufficient to induce tumorigenesis, since the replicative barrier imposed by the senescent state must first be overcome to cause transformation. In general terms, senescence is considered to be irreversible under physiological conditions and without additional genetic lesions; it has not fully been demonstrated that in vivo senescent cells can re-enter the cell cycle under physiological conditions. However, in vitro studies have shown that replicative senescence reversal can occur when the expression of $p16^{INK4a}$ is low and p53 is inactivated [120], suggesting a potential scenario for reversal of senescence in vivo; indeed, $p16^{INK4a}$-negative senescent cells are frequently found in premalignant lesions like dysplastic nevi, [25] and non-dividing cells can also accumulate additional mutations [121]. Moreover, the accumulation of mutations in senescent cells may not be a rare event due to the deficiencies in DSB repair mechanisms discussed above [117,119]. Additionally, the pro-inflammatory local environment generated by the SASP could further induce DSB through ROS and be a promoter of genetic instability in senescent cells [122].

Interestingly, recent studies suggest that upregulation of SIRT6 can contribute to improve the age-related decline in DNA repair mechanisms. SIRT6 has been found to be able to rescue the age-related decline in base excision repair [123]. Specifically, SIRT6 reverted the decline of homologous recombination repair during replicative senescence [117]. Moreover, SIRT6 decline has been associated with induction of senescence in a model of osteoarthritis and in old chondrocytes [124,125]. These studies suggest that the genomic instability associated with aging and senescence could be reversed through the modulation of epigenetic remodeling enzymes, such as the sirtuins. Therefore, pharmacological targeting of SIRT6 and other proteins may be an interesting approach to prevent the decline in genome maintenance and, consequently, cancer development.

Even though accumulation of mutations in senescent cells in vitro or in vivo has not been directly addressed, the event that $p16^{INK4a}$-deficient senescent cells develop p53 mutations is, in principle, a possible scenario; therefore, senescence might represent a robust but not insurmountable barrier to cancer progression [20]. Moreover, as the inactivation of p53 in senescent cells causes dysregulation of the SASP [29], the hypothetical senescent cell carrying p53 mutations would be highly dangerous because in addition to the instable epigenetic state, the dysregulated SASP can drive malignancy in the neighboring cells.

6. Pseudo-senescence

Cellular senescence is a highly heterogeneous and plastic phenotype, and the existence of senescent cell subtypes has been proposed [13]. Thus, it could be speculated that, as part of this diversity, there could be rare senescent cells holding an intermediate, still undefined, state that has the potential to re-start proliferation and initiate cancer [126].

Recent studies provide interesting evidence regarding this issue. Damsky and colleagues reported that the inactivation of *Cdkn2a* and *Lkb1*, in the context of $Braf^{V600E}$ mutation, led to overcome OIS arrest and induced progression to melanoma in a mouse model, in contrast to what observed when only *Cdkn2a* was inactivated. Lkb1 can inhibit the mTOR1/2 pathway and its inhibition with miRNA99/100 prevented progression of nevi to melanomas [127]. Another study by Vredeveld and colleagues also reported that *PTEN* depletion and PI3K activation abrogated $BRAF^{V600E}$-induced senescence in human fibroblasts and melanocytes and induced tumor progression in murine $Braf^{V600E}$ nevi [128]. All this evidence suggests that OIS is not a terminal state since the growth arrest can be bypassed upon the inactivation of key tumor suppressor genes, such as *Lkb1* or *PTEN*.

These observations lead to the question of whether melanocytes are engaged in a "true" senescence program or the irreversibility of the arrest is due to a "pseudo-senescent" state. A proposed explanation is the existence of a rare subpopulation of non-senescent Braf expressing melanocytes within the nevi, which becomes evident after the induction of accelerated proliferation by the activation of the mTOR pathway [129] (Fig. 4A).

Despite the lack of strong direct evidences to support this hypothesis, few examples of pseudo-senescence have been reported. HT-p21 cells (human fibrosarcoma cell line) presented increased SA-β-gal activity as response to a simultaneous growth stimulation and cell cycle inhibition. However, treatment with rapamycin reduced the senescence markers and allowed re-entry in cell cycle, suggesting that rapamycin can delay senescence response by inducing a state very similar to senescence without irreversible cell cycle arrest [130] (Fig. 4C).

Hypoxia has been shown to cause reversible growth arrest after exposure to different senescence-inducing stimuli, preventing cells from engaging senescence also through the inhibition of the mTOR pathway [131]. Furthermore, ATM inactivation has been reported to mediate cellular metabolic reprogramming that bypasses the senescence response triggered by nucleotide deficiency and replication stress [132].

Another recent study showed that in melanomas characterized by high levels of Wnt5A, exposure to ionizing radiation induced typical senescence markers, including SA-β-gal positivity, SAHF, H3K9Me chromatin marks, and promyelocytic leukemia protein (PML) bodies. However, these cells retained their capacity to invade and colonize metastatic sites [133]. The authors concluded that the presence of uniform markers of senescence does not mean that the cell is truly senescent. However, these observations could also be attributed to the already mentioned heterogeneity in cell populations, with some of the cells being in a "pseudo-senescent" program or intermediate state that lacks the ability to induce permanent growth arrest in spite of expressing many of the typical senescence markers (Fig. 4B and C).

All these controversial studies indicate that the senescence is facing important challenges regarding the definition of the senescence state, the development of truly reliable markers and the identification and differentiation of potentially reversible intermediary cellular "pseudo-senescence" states that can lead to tumor development.

7. Are senescent cells premalignant cells?

Senescent cells accumulate in premalignant lesions but are not detectable after progression to malignancy [134]. Based on this observation, the role of senescent cells in tumor progression could be interpreted in different ways.

1) The senescence response halts progression to malignancy allowing time for the immune system or alternative mechanisms to eliminate the potentially dangerous cells, and malignant cells originate from cells that escape the senescence program.
2) The accumulation of senescent cells precedes progression to malignancy following the acquisition of mutations that allow them to start proliferation again. Even though the senescent response is able to initially stop tumor progression, additional oncogenic stimuli (ROS, inflammation, genomic instability) end up overcoming the proliferative arrest.
3) In either of the previous cases, tumorigenesis can be promoted by the SASP through a paracrine mechanism, as tissue-repair factors may stimulate proliferation of neighboring cancer cells [126].

The notion that senescent cells are premalignant is supported by the epigenetic similarities found between senescent and cancer cells. Altered DNA methylation profiles are associated with genome dysfunction, which is considered a hallmark of both aging and cancer. In a recent study, replicative senescent human cells were found to carry widespread DNA hypomethylation and focal hypermethylation. Importantly, methylation was increased at CpG islands in the promoter of genes whose silencing is associated with cancer promotion, suggesting that the DNA methylome of senescent cells might promote malignancy to escape the proliferative barrier [135]. Importantly, the hypomethylation associated with senescence occurs before the proliferation arrest, suggesting that pre-senescence represents a critical state in which cells are most vulnerable to become malignant.

Remarkably, cellular senescence is a highly dynamic and progressive process in which the properties of senescent cells continuously evolve and diversify [13]. During aging, the general decline of cellular functions might delay progression of the senescence program, allowing time for the onset of additional mutations in key genes (p53 or $p16^{INK4a}$) inducing irreversible growth arrest, which could allow pre-senescent cells to become malignant. This process could potentially be enhanced by the accumulation of senescent cells due to reduced immune senescence clearance. Additionally, the exacerbated pro-inflammatory SASP could also contribute to create an optimal microenvironment for cancer development, establishing multiple positive feedback loops that push cells forward a malignant pathway.

The immune clearance of premalignant senescent hepatocytes prevents tumorigenesis, whereas the depletion of immune cells promotes cancer progression [38]. In contrast, in a luciferase knock-in mouse model ($p16^{LUC}$) that allows in vivo tracking of senescent cells, $p16^{INK4a}$ expression increased exponentially with aging, but it did not predict cancer development, suggesting that the accumulation of senescent cells is not a principal determinant of cancer-related death during normal murine aging. However, $p16^{INK4a}$ was significantly activated in all the emerging neoplasms and surrounding stromal cells, and the luciferase signal allowed early detection in tumors as small as 1 mm^3 [93], suggesting that senescent cells may lose $p16^{INK4a}$ expression and cell cycle arrest, becoming potentially malignant. Importantly, this study also showed that tumors have the ability to induce $p16^{INK4a}$ expression in their surrounding stromal and infiltrated immune cells, indicating that local, non-cell-autonomous $p16^{INK4a}$ expression can also be activated in response to extrinsic signals present within emerging tumors.

8. Final remarks

The study by Golomb et al. shows clear evidence that the response to oncogenic stimulus changes at advanced ages, when it seems to be associated with a detrimental outcome characterized by accumulation of senescent cells, immune infiltration and progression to cancer, which may explain the increased cancer risk associated with aging [35]. This deterioration of cellular and/or organismal response to oncogenic stress could be explained by several factors such as age-related decline in the immune system function [136] or age-related impaired execution of the senescence response. A deleterious local response or the inefficient activation of the immune system can interfere with the break on cancer progression, and establish a positive feedback loop that makes the senescence response more inefficient as aging progresses.

It is unknown whether or how age-related deterioration in the immune system, dysregulation of the SASP and decline in the DNA repair capacity contribute to change the senescence response into a detrimental process or which are the molecular mechanisms underlying this hypothetical aging process. It is also unknown the relative contribution of these age-related features into the overall aging phenotype and increased cancer risk. However, these questions are critical to understand the overall contribution of cellular senescence to aging and age-related tumorigenesis. The approaches to address these questions are challenging and require sophisticated in vivo modeling, but can help identify strategies to promote immune clearance of senescent cells and prevent or induce regression of cancer. For this purpose, it is crucial to characterize the factors secreted or overexpressed by senescent cells that determine the immune response, and how these factors can mediate immune clearance or evasion, as it occurs in melanocytic nevi.

Another strategy to interfere with the aberrant behavior of senescent cells could be the modulation of features that promote cancer development. Increasing evidence suggest that different SASP components can stimulate cancer development, opening the door for the development of new pharmacological interventions to prevent cancer progression. In this regard, treatments with NSAIDs or rapamycin are able to block senescence-mediated pro-carcinogenic inflammation, preventing malignant transformation or tumor progression [37,107].

Unfortunately, several studies are based on the use of one type of senescence inducer, commonly replication or overexpression of oncogenes, and on a limited number of cells or tissues (hepatocytes, fibroblasts, etc.). Since the senescence response, and in particular the SASP, is highly specific on cell type and context, future studies should focus on this limitation to understand senescence in the context of cancer development and aging.

Finally, future studies will elucidate the process of cancer development by understanding the cell decision-making process between engaging hypothetical pseudo-senescence or true senescence responses, and by assessing whether these are parallel and independent mechanisms or one that represents an intermediary state that antecedes the other. The impact of aging on these hypothetical progression and decision-making processes still seems to be an exciting and promising research field that awaits to be fully developed.

Transparency document

The Transparency document associated with this article can be found in the online version.

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