ELSEVIER

Teaser Targeting senescent cells offers a new strategy to interfere with morbidities associated with age, and the potential of preventing or delaying aging of multiple tissues.

Therapeutic interventions for aging: the case of cellular senescence

Q2 **Abel Soto-Gamez and Marco Demaria**

University of Groningen, European Institute for the Biology of Aging (ERIBA), University Medical Center Groningen (UMCG), Groningen, The Netherlands

Organismal aging is a multifactorial process characterized by the onset of degenerative conditions and cancer. One of the key drivers of aging is cellular senescence, a state of irreversible growth arrest induced by many pro-tumorigenic stresses. Senescent cells accumulate late in life and at sites of age-related pathologies, where they contribute to disease onset and progression through complex cell and non-cell autonomous effects. Here, we summarize the mechanisms by which cellular senescence can promote aging, and we offer an extensive description of current potential pharmacological interventions for senescent cells, highlighting limitations and suggesting alternatives.

Introduction

Q3 Cellular senescence is a stress response characterized by the induction of a permanent cell cycle arrest. Senescence represents an important barrier to tumorigenesis by limiting the growth of potentially oncogenic cells, reviewed in [1]. To date, there is no single universal marker that can differentiate senescent cells from quiescent, terminally differentiated and other nonproliferating cells. Instead, multiple markers are combined to identify senescent cells including: (i) upregulation of $p16^{INK4a}$, a protein that prevents cell cycle progression from the G1 to S phases by inhibiting cyclin-dependent kinase (CDK)4 and CDK6 [2]; (ii) activation of the lysosomal enzyme senescence-associated β-galactosidase (SA-β-gal) [3]; (iii) formation of specialized domains of facultative heterochromatin that contribute to silencing of proliferation-promoting genes in senescent cells, known as senescence-associated heterochromatin foci (SAHF) [4]; and (iv) persistent signaling of the DNA damage response (DDR), as shown by the presence of p53-binding protein 1 (53BP1) and γH2AX foci [5]. Most studies rely on these markers, although other predictive markers of senescence such as a flattened morphology, absence of the proliferation marker Ki67, enlarged nuclear size, loss of nuclear high mobility group box 1 (HMGB1) and decreased expression of lamin B1 are also often described [6].

Senescence-associated growth arrest (SAGA) is accompanied by an overactive secretory phenotype known as the senescence-associated secretory phenotype (SASP) [7]. The SASP consists of numerous cytokines, growth factors, proteases and extracellular matrix components that,

Corresponding author: Demaria, M. (m.demaria@umcg.nl)

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

depending on the physiological context, can be either beneficial or deleterious. During early stages, SASP components promote the migration and infiltration of effector immune cells through the secretion of cytokines and facilitate tissue repair and remodeling by release of growth factors and proteases [8]; however, in later stages, persistent senescent cells negatively impact the surrounding microenvironment by impairing tissue homeostasis through complex cell and non-cell autonomous effects, reviewed in [9].

In a cell-autonomous manner, selected SASP components such as interleukin (IL)-6 and IL-8 can reinforce SAGA through autocrine pathways [10,11]. However, the same secreted components can act in paracrine signaling to neighboring cells, propagating the senescent phenotype and thus potentially hampering the regenerative capacity of surrounding tissue [12]. Similarly, in a non-cell-autonomous manner, SASP cytokines promote infiltration of immune cells, yet persistent signaling can result in disruptive chronic inflammation [13], a hallmark of aging and major contributor to age-related dysfunctions [13–18]. Indeed, senescent cells accumulate late in life and at sites of age-related pathologies [19–21], and genetic interventions enabling the effective clearance of senescent cells in genetically engineered animal models is sufficient to delay a number of age-related phenotypes [22–25].

Accordingly, a prolonged healthspan is obtained by pharmacological interventions using a novel class of drugs termed senolytics, used to selectively ablate senescent cells [26]. Senolytic interventions not only demonstrated the feasibility of extending healthspan but also evidenced the alleviation of a wide range of pre-existent age-related symptoms including: improved cardiovascular function, reduced osteoporosis and frailty [26]; enhanced adipogenesis, reduced lipotoxicity and increased insulin sensitivity [27]; improved established vascular phenotypes associated with aging and chronic hypercholesterolemia [28]; as well as radioprotection and rejuvenation of aged-tissue stem cells [29]. In this review, we will summarize the mechanisms by which cellular senescence can promote aging, followed by an extensive description of current potential pharmacological interventions for suppressing the SASP or selectively eliminating senescent cells (Fig. 1).

Interfering with the paracrine signaling of senescent cells

Pathways involved in SASP regulation

The senescence response can be promoted by a variety of stressors, such as activation of oncogenes, telomere shortening, genotoxic and oxidative stress. Various signaling pathways are activated in a stress-type-dependent fashion, yet they appear ultimately to converge with nuclear factor (NF)-κB signaling [30]. Similarly, specific SASP components vary largely on a cell-type-dependent manner, but key components are NF-κB-dependent proinflammatory proteins. Indeed, the canonical SASP cytokines IL-6 and IL-8 appear to be the most conserved and robustly expressed cytokines of the SASP [16,17]. Cytokines have multiple autocrine and paracrine effects that are considered pleiotropic. They positively regulate a variety of cellular functions, including immune responses, growth arrest and/or differentiation [31]. By contrast, in human malignancies they can stimulate cell migration, growth, invasion, angiogenesis and eventually promote metastasis, reviewed in [32]. Such observations partly explain the paradoxical roles for cellular senescence as a tumor suppressor and a tumor promoter.

The robust expression of IL-6 and IL-8 not only contributes to the induction of the SASP but also helps its maintenance, by activating a self-amplifying secretory program. Indeed, an autocrine positive feedback loop is initiated by the activation of transcription factors NF-κB and the CCAAT/enhancer binding protein beta (C/EBPβ), which transactivate numerous genes, including the coordinated expression of IL-6 and IL-8 as well as their respective receptors IL-6R/GP80 and IL-8RB/CXCR2 [10,11,33].

Several pathways have been identified as regulators of the SASP by influencing NF-κB at various levels. These include mammalian target of rapamycin (mTOR) [34,35], mitogen-activated protein kinase (MAPK) signaling [36], the phosphoinositide 3 kinase (PI3K) pathway [37] and GATA4/p62-mediated autophagy [38]. The mTOR complex is a key modulator of aging and age-related disease in various species [39]. The exact mechanisms by which mTOR regulates aging are unclear, but novel studies suggest a role in cellular senescence and the SASP. Indeed, the mTOR complex was shown to specifically promote the translation of the proinflammatory cytokine IL-1α [34], which is thought to be an early regulator of the SASP that subsequently engages the IL-6/IL-8 pathways. Indeed, coupling of IL-1α to its receptor (IL-1R) via juxtacrine signaling enables a positive feedback loop that stimulates their own expression through activation of IL-1 receptor-associated kinase (IRAK)1, an upstream regulator of NF-κB activity, and the transcription factor C/EBPβ [40]. Moreover, mTOR is also thought to interact with MAPK by increasing translation of the MK2 kinase (also known as MAPKAPK2), which acts by preventing the degradation of numerous SASP factor transcripts by ZFP36L1 [35]. In turn, MAPKAPK2 is itself a downstream target of p38MAPK, demonstrating that SASP can depend on the activation of additional signaling pathways [41].

Different components of insulin growth factor (IGF) signaling, such as the IGF-binding proteins IGFBP3 [42], IGFBP4 and IGFBP7 [36,43] are part of the SASP and act through autocrine/paracrine pathways to inhibit IGF signaling. Tissue plasminogen (t-PA) and PAI-1 are also observed in the secretome of senescent cells following different genotoxic stresses [37,44]. The SASP factor transforming growth factor beta (TGF-β) can reinforce senescence via paracrine and autocrine mechanisms. In a paracrine fashion, it can induce bystander senescence in neighboring cells by generating reactive oxygen species (ROS) and DNA damage, triggering chronic DDR signaling [45]. In an autocrine fashion, TGF-β signaling ensures a stable cell cycle arrest through the establishment of a positive feedback loop leading to $p15^{INK4b}$ induction, even after loss of $p16^{INK4A}$ [46].

NF-κB-based strategies to interfere with the SASP

Given the deleterious effects of some SASP components, therapeutic strategies for targeting the SASP without disturbing the growth arrest are currently being investigated (Fig. 2). Senescent cells are thought to contribute to tissue dysfunction largely through chronic inflammation. Pharmaceutical strategies using known anti-inflammatory drugs have therefore been approached as effective SASP modulators.

Glucocorticoids are a group of steroid hormones secreted from the adrenal cortex when the body senses stress [47], and include cortisol and corticosterone. Both hormones have strong anti-inflammatory activities and are consequently used in the treatment

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

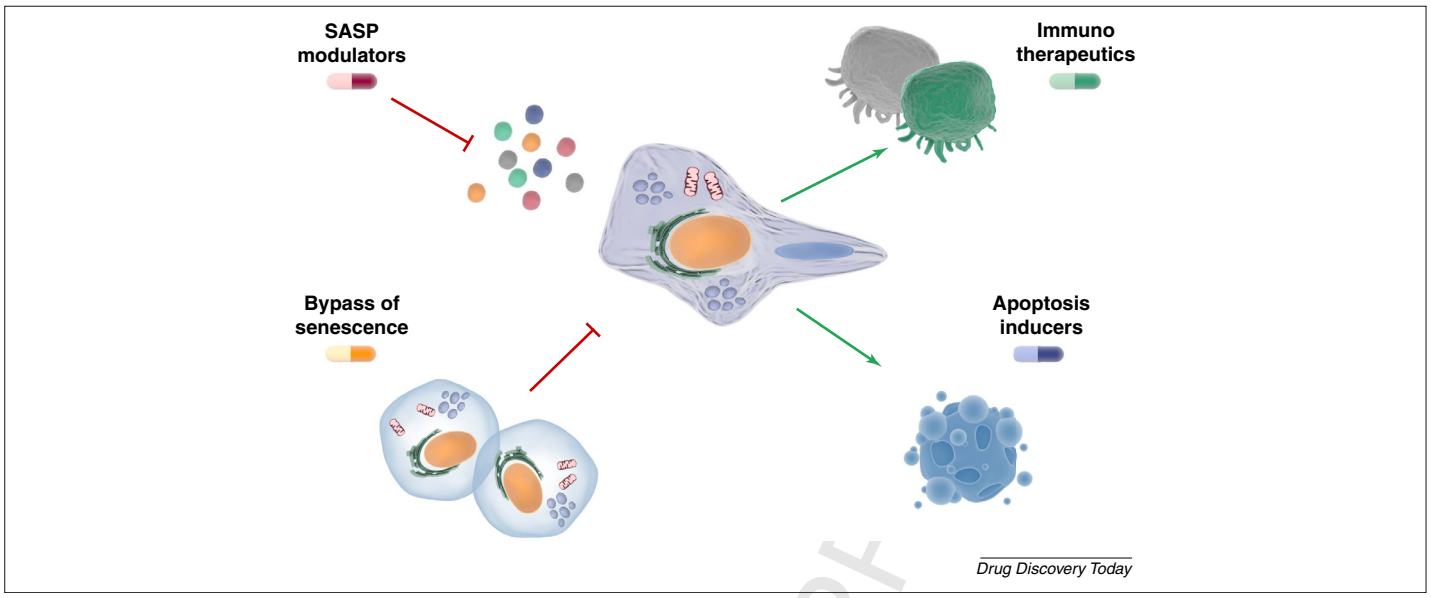

FIGURE 1

Therapeutic interventions against aging relating to cellular senescence. Various drugs interfere with the secretory phenotype of senescent cells, suggesting their clinical use for the suppression of deleterious effects associated to the senescence-associated secretory phenotype (SASP). Alternative drugs, senolytics, block pro-survival pathways active in senescent cells leading to apoptosis. Another potential approach to target senescent cells is by enhancing natural clearance by means of immunotherapy through the use of immune modulators or by increasing the number of immune effector cells. Finally, therapeutic interventions for the bypass of senescence and artificial reactivation of proliferation could possibly enhance regenerative capacity in age-related dysfunctions.

of various conditions, including asthma, allergies, autoimmune diseases and certain cancers [48]. Importantly, one of their mechanisms of action is through repression of proinflammatory cytokines [49]. Their effect on the SASP was therefore studied, demonstrating decreased secretion of selected SASP components including IL-6 [50]. The suppressive effect on the SASP was found to be largely caused by the ability of glucocorticoids to down-regulate NF-κB transcriptional activity, suggesting the FDA-approved drugs might partly exert their beneficial effects by suppressing SASP-induced inflammation. Unfortunately, side-effects and drug resistance are associated with long-term therapeutic doses of glucocorticoids, compromising their clinical applications in age-related degeneration, and possibly requiring dosage re-evaluation if intended for aging interventions.

Other commercially approved drugs have been similarly evaluated for their use as SASP regulators. Metformin (1,1-dimethylbiguanide) is a commercially available antidiabetic drug with additional effects in lowering risks of microvascular and myocardial infarction [51], cancer occurrence and general mortality, reviewed in [52]. One of the metformin modes of action involves preventing the translocation of NF-κB to the nucleus, effectively disabling activation of the NF-κB pathway [53]. An inhibitory effect of metformin on the SASP was therefore demonstrated by Moiseeva *et al.* who showed reduction in growth of prostate cancer cells otherwise fueled by SASP cytokines in the absence of metformin [54]. Similarly, prolonged tumor remission was observed in mouse xenograft cancer models upon treatment with metformin in combination with chemotherapy agents [55,56]. Lastly, as a general antiaging agent, Martin-Montalvo *et al.* demonstrated that metformin reduces oxidative stress and inflammation, extending lifespan and healthspan in mice [57]. The evidence provided by these studies has paved the way for ongoing clinical studies to examine the effects of metformin on human aging and longevity (NCT02432287).

Comparably, natural compounds, like the phenol resveratrol [58] or the flavonoids apigenin, wogonin and kaempferol, have also been shown to dampen the SASP, probably through their interaction with IκB kinases [59]. To identify novel downstream effector kinases in cellular senescence, Ferrand *et al.* screened a library of activated kinases, successfully identifying several kinases where constitutive expression induced known markers of senescence. Remarkably, the researchers also demonstrated that the kinases with the strongest pro-senescence effects induced the expression of SASP genes through activation of NF-κB [60]. However, NF-κB covers multiple functions, and its dysregulation might lead to severe consequences [61]. Identifying the specific context in which each kinase is activated is a key task for the design of less toxic interventions to limit the SASP.

A potential pharmacological alternative to dampen the SASP resides in modulating the upstream regulators of NF-κB activity. Indeed, using anti-IL-1α neutralizing antibodies or recombinant IL-1R antagonists, Orjalo and colleagues effectively disrupted the Q4 autocrine IL-1α–NF-κB positive feedback loop, markedly reducing the secretion of the canonical SASP cytokines IL-6 and IL-8 [40]. In the same way, because mTOR regulates the expression of membrane-bound IL-1α in senescent cells, selective mTOR inhibitors like rapamycin can also interfere with the IL-1α–NF-κB loop controlling much of the SASP [34]. Because rapamycin prevents the permanent loss of proliferative potential in arrested cells, without inducing proliferation [62], it can act by allowing cells to stop cell cycle progression in the face of a stressor, yet effectively suppressing SASP components that would otherwise hold arrested cells in an irreversible SAGA, or potentially propagate the senescence phenotype. Rapamycin and several analogs (known as rapalogs)

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

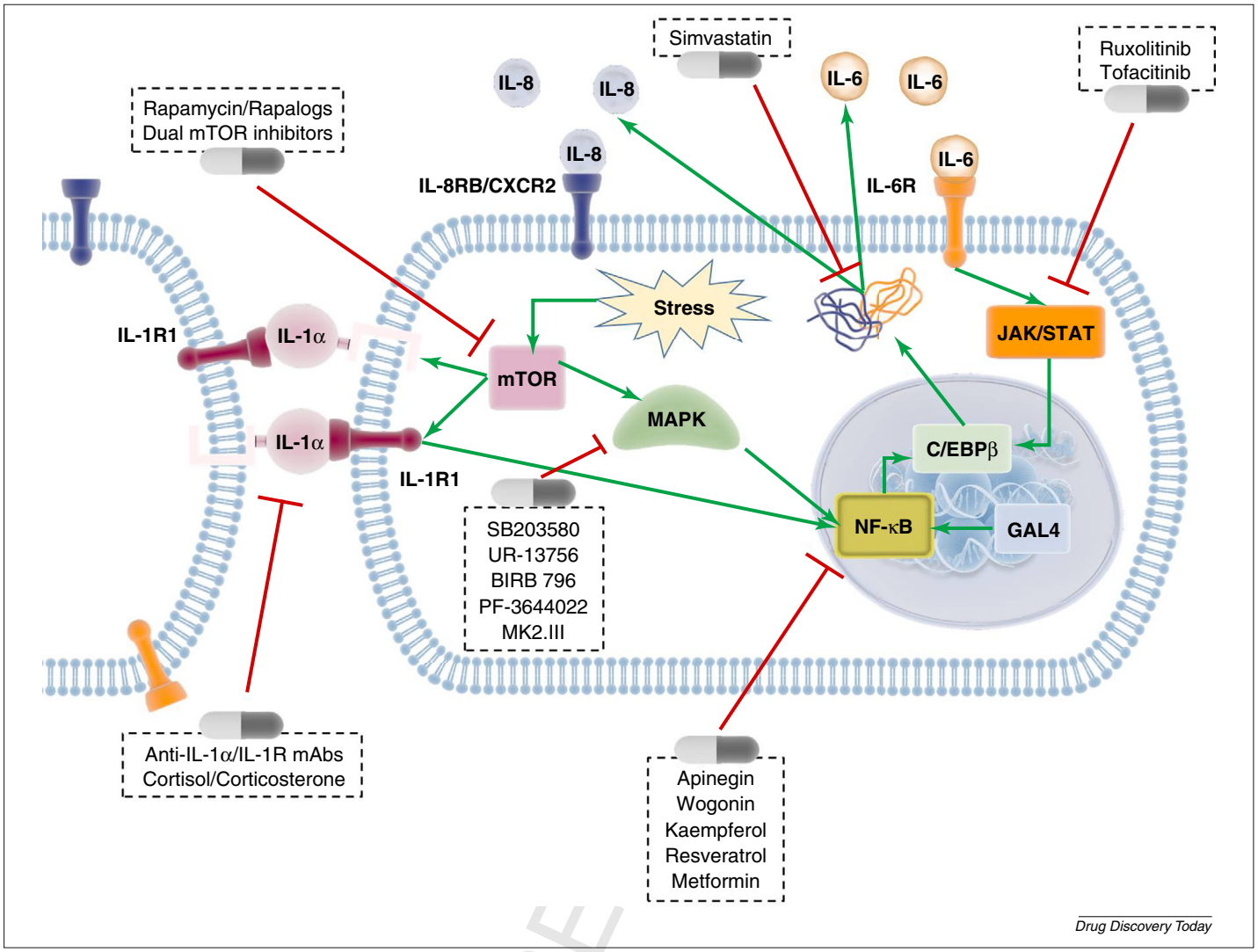

FIGURE 2

Senescence-associated secretory phenotype (SASP). Various drugs have been shown to lower production or secretion of several SASP factors. In particular, compounds that interfere with the nuclear factor (NF)-κB, Janus kinase (JAK)/signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) pathways are currently the most effective.

selectively inhibit the mTOR complex 1 (mTORC1). By contrast, 'non-rapalog' dual mTORC1/C2 inhibitors act in a broader spectrum, simultaneously targeting mTORC1 and mTORC2 complexes [63]. Alternatives to rapamycin with superior pharmacological properties have therefore been suggested as antiaging therapeutics [64,65].

The interaction of mTOR with the MAPK pathway, and DDR-independent SASP regulation through MAPK signaling, has prompted members of the phosphorylation cascade to be considered as alternative pharmaceutical targets. The p38MAPK inhibitor SB203580 and the more-specific next-generation inhibitors UR-13756 and BIRB 796 all markedly suppressed SASP expression in senescent cells [41,66]. Comparably, the p38 downstream MAK2 kinase (MAPKAPK) inhibitors PF-3644022 and MK2.III were also effective in dampening the SASP. Among these, BIRB 796 has already reached Phase III clinical trials, suggesting its possible use to suppress the SASP *in vivo* [66].

NF-κB-independent strategies to interfere with the SASP

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is a major regulator of cytokine production. In senescent cells, JAK/STAT signaling is thought to sustain the IL-6 autocrine positive feedback loop that helps reinforce senescence; binding of IL-6 to its receptor is thought to signal via JAK/STAT to activate the transcription factor C/EBPβ [67], which then drives expression of IL-6 and IL-8 [10]. Many SASP factors such as IL-6, MCP-1, vascular endothelial growth factor (VEGF) and type I/II interferons are also activators of the pathway [68]. Thus, reprograming of the SASP using JAK inhibitors has been investigated in cancer and age-related dysfunctions [69]. In oncology, enhanced chemotherapy efficacy was demonstrated using a JAK2 inhibitor [70], whereas positive effects in various age-related symptoms including reduced systemic inflammation, enhanced physical capacity, metabolic function, preserved fat tissue homeostasis, improved muscle stem cell function, muscle regeneration and

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

hair growth promotion have been described for inhibitors like ruxolitinib (JAK1/2) [27] and tofacitinib (JAK1/3), reviewed in [69].

An additional approach for NF-κB-independent SASP disruption appears to be through inhibition of protein prenylation, a post-translational modification required by key SASP components [71]. Statins are a group of cholesterol-lowering drugs that act by inhibiting the first and rate-limiting enzyme of the mevalonate pathway (HMG-CoA, HMGCR), thereby reducing cholesterol formation, as well as formation of isoprenoid intermediates essential for protein prenylation. The inhibitory effects of statins in protein prenylation could thus be exploited in dampening the SASP, and perhaps partly explaining the anti-inflammatory effects observed in selected statins [72,73]. Simvastatin treatment reduces the expression of proinflammatory cytokines such as IL-6, IL-8 and monocyte chemoattractant protein (MCP)-1 *in vitro* and *in vivo* [74,75] and can effectively suppress the effects of the SASP in fueling cancer proliferation [76]. Interestingly, known prenylated proteins include several protein kinases, signal transduction switches of the Ras superfamily and the nuclear lamina protein lamin A, which is implicated in the pathogenesis of Hutchinson–Gilford progeria syndrome. The use of prenyltransferase inhibitors is therefore actively investigated in progeria, cancer and aging [71].

Finally, other alternatives to counteract deleterious effects of the SASP include the use of neutralizing agents to block or sequester selected SASP components, thus hampering their biological action. For instance, IL-6 and IL-8 have important autocrine roles in senescence maintenance, as well as in the induction of tumor-promoting phenotypes; other SASP factors such as VEGF support increased angiogenesis [77], or act as immunosuppressants (TGF-β) [78]. These effects make selected SASP components interesting therapeutic targets. Of note, monoclonal antibodies directed against IL-6 [79], IL-8 [80], VEGF [81] or TGF-β [82] are already in development or approved for the market for the treatment of various malignancies. However, their direct effect on the accumulation of senescent cells *in vivo* remains to be tested. Similarly, additional SASP factors, such as secreted proteases and matrix-remodeling enzymes, also participate in tissue structure disruption and are key regulators in cancer and inflammation [83]; the administration of synthetic inhibitors to hamper these effects would therefore represent an interesting alternative.

Natural clearance of senescent cells

To prevent deleterious effects stimulated by their persistence, increasing evidence suggests the immunogenic phenotype of senescent cells also consists of the upregulation of surface ligands not normally expressed on healthy tissue. Indeed, natural killer (NK) cells and subsets of T cells trigger cytolytic responses on senescent cells. This phenomenon, termed senescence surveillance [84], has been demonstrated in the liver and appears to be mediated by activation of the NKG2D receptor [85–88].

The NKG2D receptor recognizes ligands on the surface of stressed cells leading to direct cytotoxicity [89–91]. NKG2D ligands are poorly expressed in normal cells, but are frequently upregulated in stressed, precancerous and tumor cells, as well as some infected cells [92]. The mechanisms leading to ligand upregulation are still unknown, but experimental evidence suggests the involvement of the DDR pathway [93]. Furthermore, similar to senescent cells propagating the senescence phenotype via SASP cytokines to neighboring cells [12], cytokine exposure and Toll-like receptor (TLR) stimulation also induces NKG2D ligand transcription [92]. It is therefore hypothesized that NKG2D ligand expression is tightly regulated in healthy adult tissue to prevent self-recognition and autoimmune reactivity. However, in contrast to adult tissue, NKG2D ligand transcripts are expressed in certain embryonic tissues, yet are undetectable post-birth [94,95]. This pattern could coincide with the role of cellular senescence in embryonic development, where developmentally programmed senescence occurs at multiple sites during embryogenesis, contributing to the patterning of mammalian embryos and facilitating tissue remodeling [96,97].

Although senescent cells can be effectively cleared in young organisms, it is possible the decreased production of immune cells during aging (immunosenescence) could reduce an aged organism's ability of carrying effective senescence surveillance. A decline in immune function with age is consistent with the high numbers of senescent cells observed at old age [13]. Additionally, the accumulation of senescent cells and their SASP factors could form a milieu permissive for the retention of senescent cells, an analog to the immunosuppresive microenvironment observed in tumors. Because of this, therapies that boost the immune system by increasing the number of immune cells, or their activity against senescent cells, could help older patients in aiding senescence surveillance.

Although neither a specific nor universal surface marker(s) has been reported for senescent cells, the selective expression of NKG2D ligands in many tumor cell lines and primary tumors has made them emerge as promising targets in oncology. Adoptive therapies involving the transfer of immune cells targeting NKG2D ligands have demonstrated therapeutic potential leading to long-term improved outcomes in cancer patients [98–101]. Nevertheless, adoptive transfer therapies can lead to life-threatening adverse effects, whereas additional downsides also limit their use in the clinic, including unfeasible times and costs dedicated to T cell culture for reaching large enough numbers of cells, the need to enhance T cell memory and effector characteristics to achieve longer persistence and an adequate selection of target antigens [102]. Finally, directly targeting the NKG2D receptor or its ligands is an alternative approach under investigation. Strategies employing monoclonal antibodies or bispecific proteins to simultaneously target NKG2D ligands in target cells and effector immune cells show therapeutic potential in oncology [92], suggesting their use could be explored for the development of senotherapeutics.

Selective elimination of senescent cells

Senescent cells make use of various pro-survival mechanisms to remain viable following DNA damage while simultaneously hampering growth. For instance, much like cancer cells, senescent cells have an active DDR and rely on antiapoptotic pathways to persist in tissues. Such mechanisms include the PI3K pathway, involved in survival regulation [103], the Bcl-2/Bcl-xL pathways which regulate mitochondrial-dependent apoptosis [104] and the blockage of dependence receptors that normally promote apoptosis [105]. These features make senescent cells much more reliant on pro-survival pathways than their nonsenescent counterparts, serving as the rationale behind the development of senolytic drugs,

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

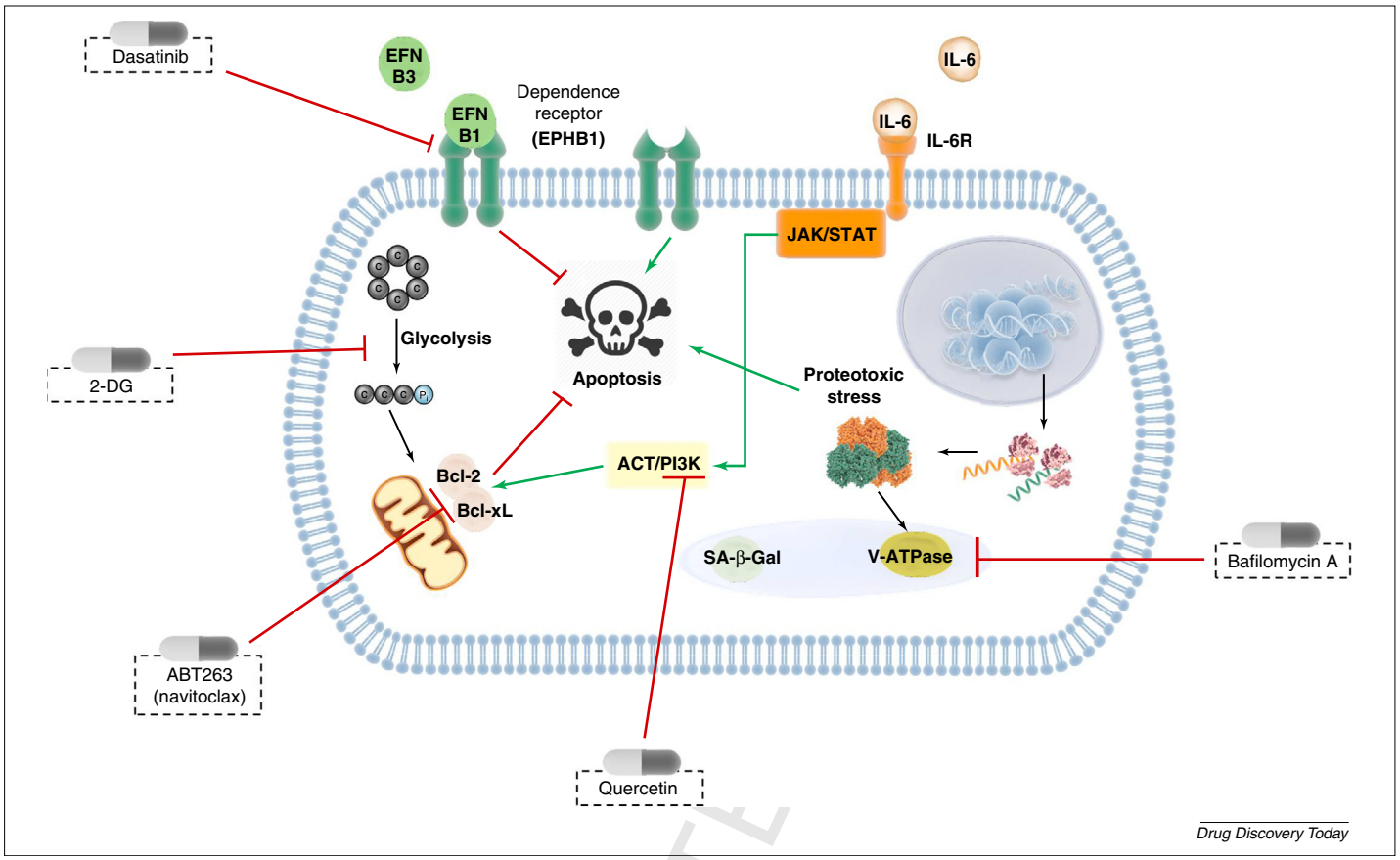

FIGURE 3

Q8 Elimination of senescent cells. Various drugs have been shown specifically to lead senescent cells to apoptosis. Particularly, interference with the phosphoinositide 3 kinase (PI3K), Bcl and metabolic pathways seems the most effective, but still effectiveness is highly cell-type dependent.

which aim to eliminate senescent cells without affecting quiescent and proliferating cells [26].

A number of senolytic drugs have now been identified (Fig. 3), including quercetin (which inhibits the PI3K pathway), dasatinib (which interferes with dependence receptor EFNB) and ABT263 or navitoclax (which targets the Bcl-2/Bcl-xL proteins) [26,106]. These drugs report a wide range of beneficial effects for senescence-related indications *in vitro* and *in vivo*; most notably enhanced cardiovascular function, improved exercise endurance, reduced osteoporosis and frailty [26,106], as well as radioprotection and rejuvenation of the hematopoietic system in mice [29]. Although promising, senolytics described to date are not effective in all senescent cell types, and must instead be tested in each senescent cell type of interest. Dasatinib effectively eliminated senescent human fat cell progenitors, yet was much less effective on senescent human umbilical vein cells (HUVECs). The opposite phenomenon was observed with quercetin, a natural polyphenol that works as a potent antioxidant and metal ion chelator [107]. Indeed, quercetin induced cell death of senescent HUVECs to a greater extent than that of proliferating cells; however, it was much less effective on targeting pre-adipocytes [26]. In the same way, the more recently described Bcl-2/Bcl-xL inhibitor navitoclax was initially suggested as a broad-spectrum senolytic, with lethal effects in HUVECs, IMR90 human lung fibroblasts and murine embryonic fibroblasts (MEFs) [106]. However, navitoclax had no effect on senescent pre-adipocytes, arguably the most abundant type of senescent cells, in humans [108].

Also challenging, despite the apparent cell-type dependency, the targeted antiapoptotic pathways might not be specific enough and can be expressed in off-target cell-types, contributing to unwanted toxicities. For example, transient thrombocytopenia and neutropenia are well-known side-effects reported upon administration of navitoclax [109]. These side-effects could in fact relate to the importance of Bcl-2 in the survival of lymphocytes and platelets in a normal setting, such as preventing the activation of apoptosis that normally follows induction of double-strand breaks during genetic recombination events [110]. A similar response might occur in melanocytes, following the UV-induced DNA damage they must endure to produce melanin. In fact, Bcl-2 has an indispensable role in the survival of melanocyte stem cells [110], and benign and malignant melanocytic nevi often overexpress the same antiapoptotic pathway [111,112].

Bcl-2 is expressed in numerous human cancers, and was identified as a drug target over the past decades. In recent years, more-selective Bcl-2 inhibitors are under development, for instance those directed against the specific BH4 domain represent novel strategies for lowering toxicity [113]. Alternatively, metabolic differences between senescent and nonsenescent cells could also be exploited for the development of senolytics. Certainly, similar to cancer cells, senescent cells display a heightened metabolic flux, which includes enhanced uptake of amino acids, higher protein quantity and synthesis rates, and strong skewing of transcripts toward genes linked to protein maturation and protein processing [114,115]. An augmented protein production is explained, at least

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

partly, by the increased secretory phenotype of senescent cells. Moreover, enhanced glucose uptake for aerobic glycolysis is a well-recognized hallmark of cancer cells shared with therapy-induced and oncogene-induced senescent cells [115]. Importantly, senescent cells not only display a hypermetabolic condition but also rely on it for survival, as demonstrated by selectively susceptible death of therapy-induced or oncogene-induced senescent cells using 2-DG, a false substrate for glycolytic metabolism [115].

In addition to higher energy consumption, increased protein production also exerts important proteotoxic stress, causing senescent cells to depend on metabolic compensatory mechanisms. In fact, senescent cells also rely on autophagy and an intact lysosomal protein degradation machinery to relieve proteotoxic stress by degrading improperly folded, potentially toxic proteins [115]. Using specific inhibitors of lysosomal V-ATPases (bafilomycin A1 or concanamycin A), or a cocktail of lysosomal protease inhibitors, Dorr *et al.* demonstrated higher metabolic death of therapy induced senescent cells compared with nonsenescent cells, and improved survival of chemotherapy-treated mice bearing lymphomas [115]. Put together, these findings highlight the opportunity of using metabolic targeting for selective elimination of senescent cells. However, given the oncologic background of the cells tested, the selective susceptibility of senescent cells derived from alternative inducers such as replicative or paracrine senescence remains to be evaluated.

Reactivation of cell proliferation

Aging tissues display a progressive decline in regenerative capacities leading to age-dependent loss of organ function and impaired homeostasis. These changes have been attributed to degenerative changes in tissue-specific stem cells and their niches [116]. Muscle weakness and frailty correlate with a higher incidence of cells expressing senescent markers. Impaired stem cell self-renewal has been linked to replicative senescence, as a direct consequence of telomere shortening, which activates DDR signaling eventually leading to pRB/$p16^{INK4a}$ activation and SAGA [117]. Additional studies confirm $p16^{INK4a}$ promotes aging phenotypes in stem cells, whereas its silencing can enhance the regenerative potential of brain and bone marrow stem cells [118,119].

The reactivation of cell proliferation in senescent cells would therefore appear of interest for improving the regenerative capacity of aged tissues. Although growth arrest in senescent cells is considered essentially irreversible, genetic and pharmacological strategies have shown SAGA can be overridden. The upregulation of telomerase, for instance, reverses telomere attrition and effectively bypasses senescence as shown *in vitro* [120], whereas the ectopic expression of chromodomain-containing protein 8 (CBX8), a polycomb group protein that regulates proliferation through direct binding to the $p16^{INK4a}$ locus, also leads to bypass of senescence and cellular immortalization [121].

Growth arrest is reinforced in an autocrine manner by the SASP cytokines IL-6 and IL-8 [12]. IL-8 is recognized by the chemokine receptor CXCR2 which is upregulated on the surface of senescent cells and preneoplastic lesions. Thus, using a selective and competitive inhibitor of CXCR2 (SB225002), Acosta *et al.* could bypass oncogene-induced senescence *in vitro* [10]. Comparably, neutralizing antibodies against IL-6 or knockdown of IL-6 or its receptor (IL-6R) also result in bypass of senescence [11]. Of interest, Kuilman and colleagues additionally showed the transcription factor C/EBPβ was recruited to the promoters of IL-6 and IL-8 in response to oncogenic stress [11], suggesting C/EBPβ might be an interesting target for overcoming growth arrest.

Retroviral-based functional genetic screens have identified several genes regulating bypass of senescence and immortalization (either by gain or loss of function). These include inactivation of cell-cycle regulatory genes such as $p16^{INK4a}$, $p21^{Cip1/Waf1}$, p53, pRB or PPP1CA; and overexpression of oncogenic protein/transcription factors such as Klf4, c-Myc, Bmi-1 or viral oncogenes [122]. However, most of these genes are widely altered in human tumors and are most probably implicated in their causality.

More recently, Abad *et al.* demonstrated *in vivo* reprogramming of adult cells was capable of restoring their proliferative potential. Reprogramming was achieved through the transitory induction of the four transcription factors Oct4, Sox2, Klf4 and c-Myc in mice, which led to the generation of induced pluripotent stem cells *in situ*, demonstrating reprogramming can be performed within tissues [123]. These findings are relevant for future applications in regenerative medicine, and partly indicate similar approaches could restore proliferation in senescent cells or in cells that have been arrested by non-cell-autonomous effect.

Potential side-effects of interfering with senescent cells

Cell autonomous

Cellular senescence was initially identified as a permanent growth arrest in cells where multiple replication cycles gradually resulted in telomere attrition. Replicative senescence was therefore proposed as a cell-autonomous tumor-suppressive mechanism disabling cell division in cells that would otherwise result in aberrant chromosomal recombination and genomic instability. Accordingly, the same role is defined in oncogene-induced senescence and therapy-induced senescence, where overcoming growth arrest in cells with widespread DNA damage or oncogenic activation normally results in hyperplastic anomalies. In fact, virtually all human cancers lack functional $p53/p21^{Cip1/Waf1}$ and/or $RB/p16^{INK4a}$ pathways, in addition to often carrying mutations in genes known to collaborate *in vitro* to bypass the senescence response [122]. Overcoming SAGA of senescent cells should therefore be approached with caution.

In genetically modified mice enabling the drug-inducible reprogramming of adult cells *in situ*, reprogramming appeared capable of restoring proliferative potential in multiple tissues. However, the interventions came at an elevated price, resulting in multiple teratomas within multiple organs [123]. Interestingly, such effects were not observed in genetically modified mice enabling drug-inducible suicide of senescent cells [22–24] suggesting elimination of senescent cells is perhaps preferable to the bypass of senescence.

Non-cell autonomous

Contrary to the cell-autonomous role of the SASP, non-cell-autonomous effects via the SASP are much less clear and context dependent. For instance, cellular senescence has a recognized role facilitating wound healing. In genetic models allowing continuous drug-induced removal of senescent cells, delayed wound healing was observed until the drug stimulus was removed, and partially rescued upon topical treatment with recombinant platelet-derived growth factor (PDGF)-AA [8].

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

Additionally, senescent cells have an important role in the limitation of fibrosis. For instance, cellular senescence limits fibrosis by halting the proliferation of stellate cells responsible for the production of extracellular matrix deposited in fibrotic scar, and contributes to attract immune cells facilitating wound healing and fibrosis reversion by secreting extracellular-matrix-degrading enzymes and SASP components [87]. Suppression of key SASP components could therefore lead to anomalies in immune cell recruitment during wound healing or even tumor surveillance. For instance, inhibition of NK cells delays fibrosis resolution [87]; whereas a reduction in neutrophil recruitment is observed upon treatment with a CXCR2 antagonist (SB225002) in an experimental colitis model. However, neutrophil influx only appears critically dependent on CXCR2 in the early phases (8 h), but not in the later phases (day 7) [124]. Intermittent interventions for eliminating senescent cells should therefore allow for removal of chronic persistent cells, without interfering with normal immune cell recruitment post-treatment.

Although mice lacking key senescence regulators displayed excessive fibrosis upon liver injury [85], no effects were observed in drug-inducible models, perhaps because the senescence response drove instead to caspase-mediated apoptosis, thus limiting the proliferation of stellate cells [24]. Intriguingly, in some contexts, chronic senescence can contribute to a fibrotic pathology rather than ameliorate it, as observed in idiopathic pulmonary fibrosis (IPF), a progressive and fatal lung disease. In IPF fibroblasts Nox4 mediates senescence and apoptosis. Genetic and pharmacologic targeting of Nox4 led to a reversal of persistent fibrosis in aged mice models with a stablished fibrosis phenotype [125]. The direct effects of senolytic interventions in fibrosis thus remain unclear. In the same way, loss of senescence in developmentally programmed senescence was partially compensated by apoptosis but still resulted in detectable developmental abnormalities [96]. As with most pharmaceuticals, senolytic interventions would therefore be most probably avoided during pregnancy to prevent miscarriage or fetal malformation.

In oncology, strategies to suppress the SASP could in theory interfere with SAGA and even impair tumor surveillance, resulting in higher cancer susceptibility. Fortunately, however, pharmacological interventions reducing the SASP do not appear to fuel tumor progression, and instead show an opposite effect, prompting their use in cancer therapies. In fact, many described senolytics were initially identified as anticancer drugs [26], and clinical trials have been conducted with many of them, including Bcl-2 inhibitors (e.g., dasatinib, navitoclax) [126] and mTOR inhibitors such as rapamycin (sirolimus) and rapalogs including RAD001 (everolimus), CCI-779 (temsirolimus) and AP23573 (deferolimus), to evaluate their anticancer efficacy [127]. Even more so, the combination of mTOR inhibitors with a Bcl-2 antagonist with potential senolytic activity (BDA-366) exhibits exceptional synergistic effects against lung cancer [128], markedly questioning deleterious roles for senolytic interventions in tumor progression.

Concluding remarks and future perspectives: pharmacological interventions in humans

Although regeneration capacity deteriorates with age in mammals, it remains intact in other species such as salamanders. Surprisingly, salamanders show a significant induction of cellular senescence during limb regeneration; however, rapid and effective mechanisms of senescent cell clearance operate in regenerating tissues. Accordingly, the number of senescent cells does not increase upon aging, in contrast to mammals [129]. However, very recently senescent cells have been shown to promote tissue regeneration also in mammals, probably through secretion of specific SASP factors (Serrano Science). Thus, pharmacological or localized assisted immunological clearance of senescent cells might potentially aid regeneration of dysfunctional aged tissues.

The various beneficial effects resulting from the administration of drugs to selectively eliminate senescent cells, or suppress the deleterious aspects of the SASP, encourage their use in the treatment of age-related disabilities and chronic diseases as a group. Unfortunately, many challenges are still to be overcome for a successful drug development program, including increased selectivity and reduction of off-target effects. The optimization of therapeutic dosage in already approved drugs, now repurposed for aging interventions, appears promising in the reduction of unwanted side-effects, as demonstrated for rapamycin using lower intermittent doses [130]. Additionally, the development of appropriate animal models capable of demonstrating the beneficial effects using clinically relative outcomes is imperative [131]. These models would ideally be capable of distinguishing on-target from off-target effects to enable a correct assessment of safety and efficacy at a preclinical level, and ultimately grant their use in human clinical trials. In the near future, it is most likely that interventions against cellular senescence will only be prescribed on a case-by-case basis, for specific age-related dysfunctions, in patients with a favorable risk:benefit tradeoff; as is already the case in oncology where many identified senolytics are currently under investigation. Promisingly, however, human clinical trials are already underway to evaluate pharmaceutical impacts on longevity and human aging as a whole, extending our understanding on the human biology of aging and suggesting antiaging interventions could be closer than expected.

References

1 Loaiza, N. and Demaria, M. (2016) Cellular senescence and tumor promotion: is aging the key? *Biochim. Biophys. Acta* 1865, 155–167
2 Sherr, C.J. and Roberts, J.M. (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* 13, 1501–1512
3 Dimri, G.P. *et al.* (1995) A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 92, 9363–9367
4 Narita, M. *et al.* (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* 113, 703–716
5 Zhang, R. *et al.* (2005) Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev. Cell* 8, 19–30
6 Sharpless, N.E. and Sherr, C.J. (2015) Forging a signature of *in vivo* senescence. *Nat. Rev. Cancer* 15, 397–408
7 Coppé, J.P. *et al.* (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.* 5, 99–118
8 Demaria, M. *et al.* (2014) An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev. Cell* 31, 722–733
9 Ovadya, Y. and Krizhanovsky, V. (2014) Senescent cells: SASPected drivers of age-related pathologies. *Biogerontology* 15, 627–642
10 Acosta, J.C. *et al.* (2008) Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* 133, 1006–1018

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

11 Kuilman, T. *et al.* (2008) Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133, 1019–1031
12 Acosta, J.C. *et al.* (2013) A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* 15, 978–990
13 van Deursen, J.M. (2014) The role of senescent cells in ageing. *Nature* 509, 439–446
14 Ferrucci, L. *et al.* (2005) The origins of age-related proinflammatory state. *Blood* 105, 2294–2299
15 Franceschi, C. (2007) Inflammaging as a major characteristic of old people: can it be prevented or cured? *Nutr. Rev.* 65, S173–S176
16 Davalos, A.R. *et al.* (2010) Senescent cells as a source of inflammatory factors for tumor progression. *Cancer Metastasis Rev.* 29, 273–283
17 Freund, A. *et al.* (2010) Inflammatory networks during cellular senescence: causes and consequences. *Trends Mol. Med.* 16, 238–246
18 Franceschi, C. and Campisi, J. (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A Biol. Sci. Med. Sci.* 69 (Suppl. 1), S4–S9
19 Jeyapalan, J.C. *et al.* (2007) Accumulation of senescent cells in mitotic tissue of aging primates. *Mech. Ageing Dev.* 128, 36–44
20 Ressler, S. *et al.* (2006) p16INK4A is a robust *in vivo* biomarker of cellular aging in human skin. *Aging Cell* 5, 379–389
21 Zhou, S. *et al.* (2008) Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. *Aging Cell* 7, 335–343
22 Tchkonia, T. *et al.* (2013) Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J. Clin. Invest.* 123, 966–972
23 Baker, D.J. *et al.* (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 479, 232–236
24 Baker, D.J. *et al.* (2016) Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* 530, 184–189
25 Xu, M. *et al.* (2015) Targeting senescent cells enhances adipogenesis and metabolic function in old age. *eLife* 4, e12997
26 Zhu, Y. *et al.* (2015) The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 14, 644–658
27 Xu, M. *et al.* (2015) JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc. Natl. Acad. Sci. U. S. A.* 112, E6301–E6310
28 Roos, C.M. *et al.* (2016) Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell* 15, 973–977
29 Chang, J. *et al.* (2016) Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat. Med.* 22, 78–83
30 Chien, Y. *et al.* (2011) Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. *Genes Dev.* 25, 2125–2136
31 Hong, D.S. *et al.* (2007) Interleukin-6 and its receptor in cancer: implications for translational therapeutics. *Cancer* 110, 1911–1928
32 Anestakis, D. *et al.* (2015) Mechanisms and applications of interleukins in cancer immunotherapy. *Int. J. Mol. Sci.* 16, 1691–1710
33 Rodier, F. *et al.* (2009) Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat. Cell Biol.* 11, 973–979
34 Laberge, R.M. *et al.* (2015) MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nat. Cell Biol.* 17, 1049–1061
35 Herranz, N. *et al.* (2015) mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat. Cell Biol.* 17, 1205–1217
36 Wajapeyee, N. *et al.* (2008) Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. *Cell* 132, 363–374
37 Kortlever, R.M. *et al.* (2006) Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat. Cell Biol.* 8, 877–884
38 Kang, C. *et al.* (2015) The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. *Science* 349, 5612
39 Harrison, D.E. *et al.* (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392–395
40 Orjalo, A.V. *et al.* (2009) Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. *Proc. Natl. Acad. Sci. U. S. A.* 106, 17031–17036
41 Freund, A. *et al.* (2011) p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J.* 30, 1536–1548
42 Elzi, D.J. *et al.* (2012) Plasminogen activator inhibitor 1 – insulin-like growth factor binding protein 3 cascade regulates stress-induced senescence. *Proc. Natl. Acad. Sci. U. S. A.* 109, 12052–12057
43 Severino, V. *et al.* (2013) Insulin-like growth factor binding proteins 4 and 7 released by senescent cells promote premature senescence in mesenchymal stem cells. *Cell Death Dis.* 4, e911
44 Özcan, S. *et al.* (2016) Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. *Aging* 8, 1316–1329
45 Hubackova, S. *et al.* (2012) IL1- and TGFβ-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine 'bystander senescence'. *Aging* 4, 932–951
46 McNeal, A.S. *et al.* (2015) CDKN2B loss promotes progression from benign melanocytic nevus to melanoma. *Cancer Discov.* 5, 1072–1085
47 Chrousos, G.P. and Kino, T. (2009) Glucocorticoid signaling in the cell. Expanding clinical implications to complex human behavioral and somatic disorders. *Ann. N. Y. Acad. Sci.* 1179, 153–166
48 Schlossmacher, G. *et al.* (2011) Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells. *J. Endocrinol.* 211, 17–25
49 Zanchi, N.E. *et al.* (2010) Glucocorticoids: extensive physiological actions modulated through multiple mechanisms of gene regulation. *J. Cell Physiol.* 224, 311–315
50 Laberge, R.M. *et al.* (2012) Glucocorticoids suppress selected components of the senescence-associated secretory phenotype. *Aging Cell* 11, 569–578
51 Holman, R.R. *et al.* (2008) 10-year follow-up of intensive glucose control in type 2 diabetes. *N. Engl. J. Med.* 359, 1577–1589
52 Gandini, S. *et al.* (2014) Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. *Cancer Prev. Res.* 7, 867–885
53 Stavri, S. *et al.* (2015) Metformin reduces the endotoxin-induced down-regulation of apolipoprotein E gene expression in macrophages. *Biochem. Biophys. Res. Commun.* 461, 435–440
54 Moiseeva, O. *et al.* (2013) Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-κB activation. *Aging Cell* 12, 489–498
55 Iliopoulos, D. *et al.* (2011) Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. *Cancer Res.* 71, 3196–3201
56 Hirsch, H.A. *et al.* (2009) Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res.* 69, 7507–7511
57 Martin-Montalvo, A. *et al.* (2013) Metformin improves healthspan and lifespan in mice. *Nat. Commun.* 4, 2192
58 Pitozzi, V. *et al.* (2013) Chronic resveratrol treatment ameliorates cell adhesion and mitigates the inflammatory phenotype in senescent human fibroblasts. *J. Gerontol. A Biol. Sci. Med. Sci.* 68, 371–381
59 Lim, H. *et al.* (2015) Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts. *Biochem. Pharmacol.* 96, 337–348
60 Ferrand, M. *et al.* (2015) Screening of a kinase library reveals novel pro-senescence kinases and their common NF-κB-dependent transcriptional program. *Aging* 7, 986–1003
61 DiDonato, J.A. *et al.* (2012) NF-κB and the link between inflammation and cancer. *Immunol. Rev.* 246, 379–400
62 Demidenko, Z.N. *et al.* (2009) Rapamycin decelerates cellular senescence. *Cell Cycle* 8, 1888–1895
63 Sousa-Victor, P. *et al.* (2015) Dual mTORC1/C2 inhibitors: gerosuppressors with potential anti-aging effect. *Oncotarget* 6, 23052–23054
64 Lamming, D.W. *et al.* (2013) Rapalogs and mTOR inhibitors as anti-aging therapeutics. *J. Clin. Invest.* 123, 980–989
65 Leontieva, O.V. *et al.* (2015) Dual mTORC1/C2 inhibitors suppress cellular geroconversion (a senescence program). *Oncotarget* 6, 23238–23248
66 Alimbetov, D. *et al.* (2016) Suppression of the senescence-associated secretory phenotype (SASP) in human fibroblasts using small molecule inhibitors of p38 MAP kinase and MK2. *Biogerontology* 17, 305–315
67 Niehof, M. *et al.* (2001) Interleukin-6-induced tethering of STAT3 to the LAP/C/EBPbeta promoter suggests a new mechanism of transcriptional regulation by STAT3. *J. Biol. Chem.* 276, 9016–9027
68 Meyer, S.C. and Levine, R.L. (2014) Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. *Clin. Cancer Res.* 20, 2051–2059
69 Xu, M. *et al.* (2016) Perspective: targeting the JAK/STAT pathway to fight age-related dysfunction. *Pharmacol. Res.* 111, 152–154
70 Toso, A. *et al.* (2015) Enhancing chemotherapy efficacy by reprogramming the senescence-associated secretory phenotype of prostate tumors: a way to reactivate the antitumor immunity. *Oncoimmunology* 4, e994380
71 Palsuledesai, C.C. and Distefano, M.D. (2015) Protein prenylation: enzymes, therapeutics, and biotechnology applications. *ACS Chem. Biol.* 10, 51–62
72 Montecucco, F. and Mach, F. (2009) Update on statin-mediated anti-inflammatory activities in atherosclerosis. *Semin. Immunopathol.* 31, 127–142

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004

73 Liou, C.J. *et al.* (2014) Oral lovastatin attenuates airway inflammation and mucus secretion in ovalbumin-induced murine model of asthma. *Allergy Asthma Immunol. Res.* 6, 548–557
74 Sakoda, K. *et al.* (2006) Simvastatin decreases IL-6 and IL-8 production in epithelial cells. *J. Dent. Res.* 85, 520–523
75 Rezaie-Majd, A. *et al.* (2002) Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. *Arterioscler. Thromb. Vasc. Biol.* 22, 1194–1199
76 Liu, S. *et al.* (2015) Simvastatin suppresses breast cancer cell proliferation induced by senescent cells. *Sci. Rep.* 5, 17895
77 Coppe, J.P. *et al.* (2006) Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. *J. Biol. Chem.* 281, 29568–29574
78 Li, M.O. and Flavell, R.A. (2008) TGF-beta: a master of all T cell trades. *Cell* 134, 392–404
79 Karkera, J. *et al.* (2011) The anti-interleukin-6 antibody siltuximab down-regulates genes implicated in tumorigenesis in prostate cancer patients from a Phase I study. *Prostate* 71, 1455–1465
80 Mian, B.M. *et al.* (2003) Fully human anti-interleukin 8 antibody inhibits tumor growth in orthotopic bladder cancer xenografts via down-regulation of matrix metalloproteases and nuclear factor-kappaB. *Clin. Cancer Res.* 9, 3167–3175
81 Shih, T. and Lindley, C. (2006) Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. *Clin. Ther.* 28, 1779–1802
82 Grütter, C. *et al.* (2008) A cytokine-neutralizing antibody as a structural mimetic of 2 receptor interactions. *Proc. Natl. Acad. Sci. U. S. A.* 105, 20251–20256
83 Coppe, J.P. *et al.* (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* 6, 2853–2868
84 Kang, T.W. *et al.* (2011) Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* 479, 547–551
85 Krizhanovsky, V. *et al.* (2008) Senescence of activated stellate cells limits liver fibrosis. *Cell* 134, 657–667
86 Radaeva, S. *et al.* (2006) Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners. *Gastroenterology* 130, 435–452
87 Sagiv, A. *et al.* (2013) Granule exocytosis mediates immune surveillance of senescent cells. *Oncogene* 32, 1971–1977
88 Sagiv, A. *et al.* (2016) NKG2D ligands mediate immunosurveillance of senescent cells. *Aging* 8, 328–344
89 Eagle, R.A. and Trowsdale, J. (2007) Promiscuity and the single receptor: NKG2D. *Nat. Rev. Immunol.* 7, 737–744
90 Iannello, A. and Raulet, D.H. (2013) Immune surveillance of unhealthy cells by natural killer cells. *Cold Spring Harb. Symp. Quant. Biol.* 78, 249–257
91 Lanier, L.L. (2015) NKG2D receptor and its ligands in host defense. *Cancer Immunol. Res.* 3, 575–582
92 Spear, P. *et al.* (2013) NKG2D ligands as therapeutic targets. *Cancer Immun.* 13, 8
93 Cerboni, C. *et al.* (2014) The DNA damage response: a common pathway in the regulation of NKG2D and DNAM-1 ligand expression in normal, infected, and cancer cells. *Front. Immunol.* 4, 508
94 Cosman, D. *et al.* (2001) ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 14, 123–133
95 Nomura, M. *et al.* (1996) Genomic structures and characterization of Rae1 family members encoding GPI-anchored cell surface proteins and expressed predominantly in embryonic mouse brain. *J. Biochem.* 120, 987–995
96 Munoz-Espin, D. *et al.* (2013) Programmed cell senescence during mammalian embryonic development. *Cell* 155, 1104–1118
97 Storer, M. *et al.* (2013) Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell* 155, 1119–1130
98 Nakajima, J. *et al.* (2010) A Phase I study of adoptive immunotherapy for recurrent non-small-cell lung cancer patients with autologous gammadelta T cells. *Eur. J. Cardiothorac. Surg.* 37, 1191–1197
99 Barber, A. *et al.* (2008) Immunotherapy with chimeric NKG2D receptors leads to long-term tumor-free survival and development of host antitumor immunity in murine ovarian cancer. *J. Immunol.* 180, 72–78
100 Barber, A. *et al.* (2011) Treatment of multiple myeloma with adoptively transferred chimeric NKG2D receptor-expressing T cells. *Gene Ther.* 18, 509–516
101 Zhang, T. *et al.* (2007) Chimeric NKG2D modified T cells inhibit systemic T-cell lymphoma growth in a manner involving multiple cytokines and cytotoxic pathways. *Cancer Res.* 67, 11029–11036
102 Wu, R. *et al.* (2012) Adoptive T-cell therapy using autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. *Cancer J.* 18, 160–175
103 Osaki, M. *et al.* (2004) PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis* 9, 667–676
104 Minn, A.J. *et al.* (1999) Bcl-xL regulates apoptosis by heterodimerization-dependent and -independent mechanisms. *EMBO J.* 18, 632–643
105 Goldschneider, D. and Mehlen, P. (2010) Dependence receptors: a new paradigm in cell signaling and cancer therapy. *Oncogene* 29, 1865–1882
106 Zhu, Y. *et al.* (2016) Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell* 15, 428–435
107 Olave, N.C. *et al.* (2010) Upstream stimulatory factor-2 mediates quercetin-induced suppression of PAI-1 gene expression in human endothelial cells. *J. Cell Biochem.* 111, 720–726
108 Tchkonia, T. *et al.* (2010) Fat tissue, aging, and cellular senescence. *Aging Cell* 9, 667–684
109 Rudin, C.M. *et al.* (2012) Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. *Clin. Cancer Res.* 18, 3163–3169
110 Mak, S.S. *et al.* (2006) Indispensable role of Bcl2 in the development of the melanocyte stem cell. *Dev. Biol.* 291, 144–153
111 Michaloglou, C. *et al.* (2005) BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 436, 720–724
112 Tsao, H. *et al.* (2003) The transformation rate of moles (melanocytic nevi) into cutaneous melanoma: a population-based estimate. *Arch. Dermatol.* 139, 282–288
113 Liu, Z. *et al.* (2016) BH4 domain of Bcl-2 as a novel target for cancer therapy. *Drug Discov. Today* 21, 989–996
114 Young, A.R. *et al.* (2009) Autophagy mediates the mitotic senescence transition. *Genes Dev.* 23, 798–803
115 Dorr, J.R. *et al.* (2013) Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. *Nature* 501, 421–425
116 Oh, J. *et al.* (2014) Stem cell aging: mechanisms, regulators and therapeutic opportunities. *Nat. Med.* 20, 870–880
117 Sacco, A. *et al.* (2010) Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice. *Cell* 143, 1059–1071
118 Sousa-Victor, P. *et al.* (2014) Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature* 506, 316–321
119 Janzen, V. *et al.* (2006) Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature* 443, 421–426
120 Pellegrini, G. *et al.* (2004) Telomerase activity is sufficient to bypass replicative senescence in human limbal and conjunctival but not corneal keratinocytes. *Eur. J. Cell Biol.* 83, 691–700
121 Dietrich, N. *et al.* (2007) Bypass of senescence by the polycomb group protein CBX8 through direct binding to the INK4A-ARF locus. *EMBO J.* 26, 1637–1648
122 Vergel, M. and Carnero, A. (2010) Bypassing cellular senescence by genetic screening tools. *Clin. Transl. Oncol.* 12, 410–417
123 Abad, M. *et al.* (2013) Reprogramming *in vivo* produces teratomas and iPS cells with totipotency features. *Nature* 502, 340–345
124 Bento, A.F. *et al.* (2008) The selective nonpeptide CXCR2 antagonist SB225002 ameliorates acute experimental colitis in mice. *J. Leukoc. Biol.* 84, 1213–1221
125 Hecker, L. *et al.* (2014) Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. *Sci. Transl. Med.* 6 231ra47
126 Harazono, Y. *et al.* (2014) Why anti-Bcl-2 clinical trials fail: a solution. *Cancer Metastasis Rev.* 33, 285–294
127 Dufour, M. *et al.* (2011) Targeting the mammalian target of rapamycin (mTOR) in cancer therapy: lessons from past and future perspectives. *Cancers* 3, 2478–2500
128 Han, B. *et al.* (2015) Small-molecule Bcl2 BH4 antagonist for lung cancer therapy. *Cancer Cell* 27, 852–863
129 Yun, M.H. *et al.* (2015) Recurrent turnover of senescent cells during regeneration of a complex structure. *eLife* 4, e05505
130 Popovich, I.G. *et al.* (2014) Lifespan extension and cancer prevention in HER-2/neu transgenic mice treated with low intermittent doses of rapamycin. *Cancer Biol. Ther.* 15, 586–592
131 Kirkland, J.L. and Tchkonia, T. (2015) Clinical strategies and animal models for developing senolytic agents. *Exp. Gerontol.* 68, 19–25

Please cite this article in press as: Soto-Gamez, A., Demaria, M. Therapeutic interventions for aging: the case of cellular senescence, Drug Discov Today (2017), http://dx.doi.org/10.1016/j.drudis.2017.01.004