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Cellular senescence in tissue repair: every cloud has a silver lining

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ABSTRACT Cellular senescence, a form of stable cell cycle arrest induced by cellular stress, constitutes a major factor leading to the promotion of pathologies and physiological decays that take place during ageing. However, in recent years evidence has started to emerge supporting a positive role for senescent cells in various physiological processes, from embryonic development to tissue injury responses such as wound healing and tissue repair. Here, we provide an overview of cellular senescence, its negative as well as positive outcomes, with a focus on its impact on tissue repair. Furthermore, we discuss the possibility that cell senescence could contribute to the regeneration of complex structures and explore recent findings with respect to their potential for therapeutic application.

KEY WORDS: *cellular senescence, development, wound healing, regeneration, salamander*

Cellular senescence is a regulated response to various forms of cellular stress whereby cells undergo a permanent cell cycle arrest, highly refractory to mitogens, as well as a series of phenotypic transformations. Initially described as a process which limits the replicative lifespan of cells in culture upon reaching the 'Hayflick limit' (Hayflick, 1965, Hayflick and Moorhead, 1961), cell senescence is now recognised as a universal anti-proliferative response to a wide range of stimuli including DNA damage, oncogene activation, telomere erosion, protein misfolding, oxidative damage and exposure to extracellular signals such as mitogens and cytokines, which can happen at any point during the lifespan of a cell (Fig. 1)(Campisi, 2013, Kuilman *et al.*, 2010, Rodier and Campisi, 2011, Serrano *et al.*, 1997, Yun, 2015). In the face of excessive or irreparable cellular and genotoxic stress, cell senescence acts as an alternative fate to apoptosis, triggering a state of permanent cell cycle arrest that prevents the propagation of compromised cells. As such, it constitutes a powerful cell-autonomous mechanism of tumour suppression (Campisi, 2005). However, unlike apoptosis, cellular senescence leads to the generation of cells which remain metabolically active within tissues and secrete a host of molecules, collectively known as the senescence-associated secretory phenotype (SASP), that can alter their microenvironment with important consequences for a wide range of biological processes from development to ageing. Through their non cell-autonomous functions, senescent cells have been shown to affect cell migration and growth, tissue architecture, cell plasticity, recruitment of immune cells and inflammatory responses. Recent evidence has linked these functions to physiologically detrimental processes such as the promotion of tumourigenesis and inflammation. Furthermore, there is mounting evidence that cellular senescence is also a major contributor to the Ageing process, driving age-related pathologies as well as age-related declines in regenerative responses (Baker *et al.*, 2016, van Deursen, 2014, Yun, 2015). Yet, at the same time, compelling evidence is emerging supporting a beneficial role for senescent cells in a variety of contexts including embryonic development (Chuprin *et al.*, 2013, Davaapil *et al.*, 2017, Munoz-Espin *et al.*, 2013, Storer *et al.*, 2013, Villiard *et al.*, 2017), wound healing (Demaria *et al.*, 2014, Jun and Lau, 2010) and additional responses to tissue injury (Kong *et al.*, 2012, Krizhanovsky *et al.*, 2008, Meyer *et al.*, 2016, Ritschka *et al.*, 2017, Yun *et al.*, 2015). Such seemingly conflicting observations could be reconciled by taking into account the phenotypic complexity of different senescent states, particularly with regard to their dynamic secretory phenotype. Understanding the molecular nature of the senescent state, its evolution, targets and regulation in space and time, will provide a base from which to elucidate the beneficial and detrimental aspects of the senescence programme and how to use this knowledge in respect of therapeutic applications in cancer, ageing and regeneration.

Abbreviations used in this paper: SASP, senescence-associated secretory phenotype.

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Molecular basis of cellular senescence

At the molecular level, the establishment of cellular senescence requires activation of classical cell cycle inhibition pathways such as p53 and/or the cyclin-dependent kinase inhibitors $p21^{CDKN1A}$, $p16^{CDKN2A/INK4A}$ and $p15^{CDKN2B/INK4B}$, critical enforcers of cell cycle arrest (Fig. 1). Activation of the p53 pathway results in the hypophosphorylation of the S-phase transition regulator retinoblastoma (Rb) and pocket proteins p130 and p107, mounting a barrier to DNA replication (Burkhart and Sage, 2008, Stein *et al.*, 1990). The CDKN2A locus plays a critical role in this process as it encodes both $p16^{INK4A}$ and another important senescence inducer, $p14^{ARF}$ ($p19^{ARF}$ in mice) which acts as a p53 stabiliser by inhibiting the E3-ubiquitin ligase that normally targets p53 for degradation, MDM2 (Kamijo *et al.*, 1998, Shay *et al.*, 1991). Yet, there are examples of senescence induction that do not depend on p53 but solely on $p16^{INK4A}$ or $p21^{CDKN1A.}$, and cases in which $p15^{INK4B}$ can take over the functions of $p16^{INK4A}$ (Krimpenfort *et al.*, 2007). The importance of these molecules as effectors of the senescence programme is highlighted by the consequences of their loss, which results in senescence bypass and tumourigenesis. Furthermore, while different senescence-promoting stimuli are known to use different signalling pathways, these ultimately converge in the activation of the cell cycle inhibition machinery. For example, senescence induced by the oncogene RAS initially involves the activation of a MAPK cascade, which results in an increase in p53 activation and p16 upregulation (Lin *et al.*, 1998). Likewise, telomere erosion and other forms of DNA damage induce senescence following DNA damage recognition and activation of the kinases ATM and ATR, which stimulates the downstream kinases CHK1 and CHK2 resulting in the phosphorylation-mediated activation of the p53/p21 axis (d'Adda di Fagagna *et al.*, 2003, Herbig *et al.*, 2004). In certain contexts, the DNA-damage response can result in expression of interferon beta (IFNβ) via ATM activation, which leads to p53 activation and senescence promotion (Yu *et al.*, 2015). Additionally, T-helper derived cytokines such as interferon gamma (IFNγ) and tumour necrosis factor (TNF) can induce a G1 senescent arrest mediated by the activation of p16 and Rb hypophosporylation (Braumuller *et al.*, 2013). Lastly, senescence induced by oncogenes such as BRAFV600E can lead to the direct activation of p16 expression (Zhu *et al.*, 1998), whereas senescence induced by the loss of the tumour suppressor PTEN is initiated through activation of mTORC pathway but eventually leads to upregulation of p16 and ARF expression (Alimonti *et al.*, 2010). The involvement of mTORC in oncogene-mediated senescence induction hints at a causal relationship between senescence and autophagy. Indeed, inhibition of autophagy has been shown to delay $HRAS^{G12V}$ induced senescence (Young *et al.*, 2009), leading to the suggestion that autophagy could contribute to the generation of metabolites to fuel the synthesis of SASP factors, facilitating senescence. More recent experiments described a role for autophagy in lamin B1 degradation (Dou *et al.*, 2016), a trigger of p53 activation which leads to proliferative arrest and senescence (Shimi *et al.*, 2011). However, recent data also suggests that inhibition of autophagy can promote, rather than impair, cellular senescence in proliferating cells (Garcia-Prat *et al.*, 2016), as discussed in the next section. These paradoxical observations were recently reconciled by data demonstrating that autophagy leads to the activation of factors that have opposite effects on cellular senescence and therefore autophagy inhibition can result in different outcomes depending on the cellular context (Kang and Elledge, 2016). In particular, selective autophagy suppresses cellular senescence through degradation of GATA4 (Kang *et al.*, 2015), a transcription factor that regulates

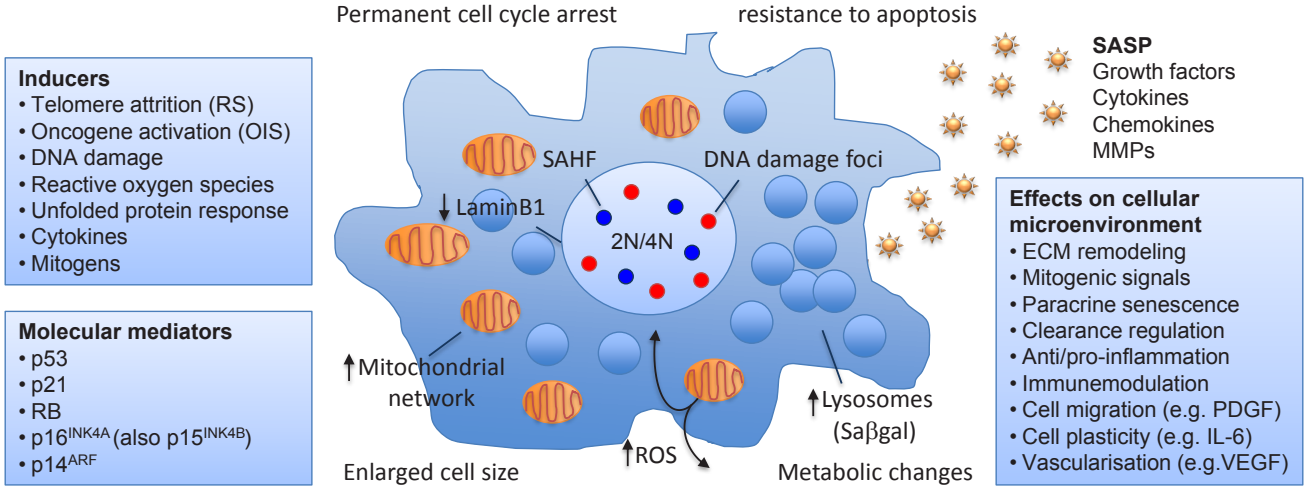

Fig. 1. Features of cellular senescence. *Senescent cells are characterised by a permanent cell cycle arrest, induced by a range of different stimuli including telomere attrition, DNA damage, oncogene activation and exposure to extracellular signals such as cytokines and mitogens. These stimuli result in the activation of key cell cycle regulators such as p53, p21, p16 and $p14^{ARF}$, leading to cell cycle exit in either G1 or G2 (with 2N or 4N DNA content) and the acquisition of phenotypic changes including apoptosis resistance, expansion of mitochondrial and lysosomal networks, increased lysosomal senescence-associated β-galactosidase (Saβgal) activity, production of reactive oxygen species (ROS), metabolic reshaping, enlarged cell size, heterochromatin changes resulting in the appearance of senescence-associated heterochromatic foci (SAHF), presence of DNA damage foci, decrease in LaminB1, and expression of a senescence-associated secretory phenotype (SASP) which includes cytokines, growth factors and matrix remodelling proteins. Manifestation of these hallmarks varies according to the nature of the senescence stimulus, the cell type and time from induction. Through the SASP, senescent cells can modify their microenvironment, with important consequences for physiological processes such as inflammation, tumourigenesis, morphogenesis and regenerative responses.*

the SASP, while general autophagy facilitates senescence through mTOR and p53 activation.

While the activation of a cell cycle inhibitory response is common to all senescent cells, the critical determinants of such inhibition vary according to the nature of the cell, the initial stimulus and even the species, both *in vitro* and *in vivo*. This is exemplified by the p21-dependent but p16-independent senescence observed in the mesonephros and apical ectodermal ridge during mouse development (Munoz-Espin *et al.*, 2013, Storer *et al.*, 2013), which contrasts with the p16-dependent senescence induced during wound healing (Demaria *et al.*, 2014) and many stem cell populations in ageing contexts (Baker *et al.*, 2016, Chang *et al.*, 2016, Sousa-Victor *et al.*, 2014). Additionally, cultured mouse embryonic fibroblasts rely on $p19^{ARF}$ for the maintenance of the senescent state, yet human fibroblasts depend largely on p16 (Collins and Sedivy, 2003, Sharpless and Sherr, 2015). Differences in molecular requirements for the induction of senescence are also found according to the stage of the cell cycle from which a cell enters senescence. Although traditionally described as an irreversible form of G1 arrest, recent studies have shown that senescence can also take place during the G2 phase of the cell cycle through p21-mediated inhibition of mitotic CDK complexes (Baus *et al.*, 2003, Gire and Dulic, 2015, Herbig *et al.*, 2004). When this mechanism fails, mitotic slippage can occur, leading to cells with 4N content which undergo a senescence arrest in the subsequent G1 phase which depends on p53/Rb for its induction but relies primarily on p16 for its maintenance (Johmura *et al.*, 2014). Hence, mechanistically, senescence induced in G2 is distinct from the G1 arrest which is caused by adaptation to the spindle checkpoint or by defects in cytokinesis (Gire and Dulic, 2015). Despite these differences, senescent cells invariably display high levels of at least one of the central cell cycle inhibitors, a feature that constitutes a molecular hallmark of the senescent state.

Hallmarks of cellular senescence

Reflecting the multiplicity of triggers and molecular pathways involved in its establishment, cellular senescence is associated with a repertoire of phenotypically diverse cellular states whose characteristics depend on the mechanism of induction, the nature of the cell and the time since the initial stimulus, and whose manifestation ultimately determines the physiological impact of the senescent cell. Yet, there are features shared by the majority of senescent states described so far (Fig. 1). The most prominent among them is a persistent cell cycle arrest that, in contrast to quiescence, is unresponsive to mitogenic signals and is governed by the molecular mediators discussed above. Indeed, this is the defining hallmark of cellular senescence. Such a robust cell cycle arrest may also be found in terminally differentiated cells, however senescent cells are characterised by a series of additional features that distinguish them from their differentiated counterparts. These include morphological changes (increase in cell volume, flattened morphology in culture), an expansion of mitochondrial and lysosomal networks (leading to high levels of senescence-associated-β-galactosidase -SAβgal- activity, a widely used senescent cell marker (Dimri *et al.*, 1995)), increases in metabolic rate, production of reactive oxygen species (ROS), a marked resistance to apoptosis, epigenetic rearrangements, persistent DNA damage foci (depending on the stimulus and frequently containing DNA damage sensors such as γH2AX and 53BP1 (Rodier *et al.*, 2011)) and the acquisition of a senescence-associated secretory phenotype (SASP) which comprises growth factors, cytokines, chemokines and matrix-remodelling proteins (MMPs) (Acosta *et al.*, 2008, Kuilman *et al.*, 2008) and is responsible for the non cell-autonomous functions of senescent cells (Coppe *et al.*, 2008). Some of these features are characteristic of certain types of senescence, while others (such as cell cycle inhibitor expression, lack of proliferation markers and SAβgal activity) are common to all.

In addition to the aforementioned hallmarks, many examples of the senescent state exhibit a spatial reorganisation of heterochromatin into senescence-associated heterochromatic foci (SAHF). This accompanies the profound alterations in gene expression seen in senescent cells (Chandra and Narita, 2013, Rai and Adams, 2013). The repressive chromatin marks tri-methylated histone 3 in lysine 9 and 27, H3K9me3 and H3K27me3 respectively, are segregated from each other within SAHF (Chandra and Narita, 2013), forming discrete spatial domains in a process that requires LaminB1 downregulation and relocalisation within the nucleus (Sadaie *et al.*, 2013). In addition, the histone variants H3.3 and macroH2A have been shown to increase during senescence, suggesting that they may play a role in the maintenance of chromatin structure within senescent cells (Rai and Adams, 2013). Such heterochromatin changes are functionally relevant, as highlighted by the requirement of H3K9me-dependent heterochromatin formation for the silencing of growth factor promoting genes mediated by Rb during oncogene-induced senescence. This process is mediated in part by the histone methyltransferase Suv391, and its failure results in tumour development (Braig *et al.*, 2005).

Another common feature of senescent cells is a marked resistance to apoptosis, which is molecularly determined by expression of pro-survival, anti-apoptotic regulators such as Bcl-Xl and Bcl-2 (Wang, 1995), downregulation of apoptotic effectors such as caspase-3 (Marcotte *et al.*, 2004), or inability to stabilise p53 to the levels required for eliciting the apoptotic programme (Seluanov *et al.*, 2001). In this connection, it is worth noting that cell senescence constitutes an alternative response to apoptosis upon DNA damage and cell stress, and that they have common molecular activators such as p53. The balance between senescence and apoptosis is determined by the cell type, the type and magnitude of the damage, and established at the molecular level by the extent of p53 expression, post-translational modifications and activation. Furthermore, interfering with the ability of a cell to undergo apoptosis results in a switch towards senescence. Moreover, downregulating or inhibiting anti-apoptotic regulators in senescent cells can make them undergo apoptosis. This is an important notion in the light of the recent development of senolytics (molecules that specifically lead to the elimination of senescent cells) such as ABT-263 and ABT-737, specific inhibitors of the anti-apoptotic proteins BCL-2 and BCL-XL which act by inducing apoptosis specifically in senescent cells (Chang *et al.*, 2016, Yosef *et al.*, 2016).

The identification of these hallmarks opened the door to the detection, characterization and targeting of senescent cells in *in vivo* contexts. Thus, detection of senescence-associated β-galactosidase enzymatic activity at pH 6 (a suboptimal pH for the activity of this lysosomal enzyme, which normally operates at pH 4–4.5, but one that allows its detection in cells with high activity such as senescent cells (Lee *et al.*, 2006)), combined with expression of $p16^{INK4A}$, $p19^{ARF}$, p21 or p53, lack of proliferation markers (eg. nucleotide analogue incorporation or decreased levels of Ki67

or PCNA), intracellular lipofuscin accumulation and expression of SASP components, have been instrumental in establishing that senescence does not simply constitute a cell culture singularity but occurs in various *in vivo* settings (Evangelou *et al.*, 2017, Yun, 2015). Furthermore, senescence-associated features have facilitated the development of functional tools, including genetic labelling and ablation cassettes based on p16 regulatory regions (Baker *et al.*, 2011, Burd *et al.*, 2013, Demaria *et al.*, 2014) and senolytic molecules (Chang *et al.*, 2016, Yosef *et al.*, 2016).

Enter the senescence-associated secretory phenotype (SASP)

Among the hallmarks of senescence, perhaps the most relevant to its diverse physiological roles is the secretory phenotype. The SASP constitutes a highly dynamic entity which develops gradually following the induction of senescence and is found in most senescent cells reported so far, both *in vitro* and *in vivo* (Coppe *et al.*, 2008, Ito *et al.*, 2017). SASP factors include growth factors (eg. TGFβ, HGF, VEGF, PDGF), cytokines (eg. interleukins such as IL-1, IL-6 and IL8), chemokines (eg. monocyte chemoattractant protein 1, MCP-1), and proteases (eg. cathepsins, matrix metalloproteinases)(Acosta *et al.*, 2013, Coppe *et al.*, 2010, Coppe *et al.*, 2008, Ito *et al.*, 2017). A hierarchy is evident among the SASP factors, as some of them are required for the maintenance whilst others for the induction of the secretory phenotype. This is the case with IL-1, which is an essential trigger of the SASP in oncogene-induced senescence (Acosta *et al.*, 2013). Expression of IL1α can activate the c/EBPβ and NFκB pathways, which cooperatively regulate SASP components in various senescence contexts (Acosta *et al.*, 2013, Kang *et al.*, 2015, Kuilman *et al.*, 2008), resulting in induction of the SASP. Other factors, such as IL-6 and CXCR2-binding chemokines, can form positive feedback loops that reinforce the expression of the SASP as well as the growth arrest (Acosta *et al.*, 2008, Kuilman *et al.*, 2008), whereas the TGFβ ligands VEFG, CCL2 and CCL20 have been shown to regulate cell cycle inhibitors p15 and p21 (Acosta *et al.*, 2013). Additionally, IL-1 and TGFβ cooperate to promote the production of ROS in senescent cells (Hubackova *et al.*, 2012). Thus, beyond the effects it may have in neighbouring cells, the SASP helps reinforcement of the senescent state.

Arguably the most prominent aspect of the SASP is its non cell-autonomous nature, which enables senescent cells to communicate with or modify their microenvironment determining their functional impact. Through the SASP, senescent cells can induce paracrine senescence in their neighbours, via a TGFβ, IL-1 and ROS-dependent 'bystander' mechanism which occurs *in vitro* and *in vivo* (Acosta *et al.*, 2013, Hubackova *et al.*, 2012, Nelson *et al.*, 2012, Yun *et al.*, 2013). In addition, the SASP underlies complex interactions between senescent cells and the immune response. For example, it can drive the recruitment and activation of immune cells, including monocytes/macrophages, NK and T-cells, leading to the subsequent elimination of senescent cells (Sagiv and Krizhanovsky, 2013). During oncogene-induced senescence, this immune surveillance mechanism has recently been shown to rely on a pro-inflammatory SASP developed at late stages following senescence induction, which is actively suppressed by Notch1 signaling at earlier stages (Hoare *et al.*, 2016). Senescent cell clearance has been shown to act as an anti-tumourigenic mechanism: senescence of pre-malignant hepatocytes promotes their clearance by CD4+T-cells and macrophages and prevents cancer progression (Kang *et al.*, 2011); likewise, induction of p53-dependent senescence in p53-/- liver carcinomas can elicit tumour regression, dependent on the clearance of the senescent cells (Xue *et al.*, 2007). Additionally, the SASP can regulate immune cells directly. In a p53-dependent manner, senescent hepatic stellate cells secrete factors that skew macrophage polarization towards a pro-inflammatory M1 type, which then contributes to tumour suppression (Lujambio *et al.*, 2013). Notwithstanding these anti-tumourigenic functions, the SASP has been shown to contribute to tumour progression through a variety of mechanisms, including the promotion of malignant cell growth in culture (Krtolica *et al.*, 2001) and in xenografts through the secretion of mitogens such as HGF (Liu and Hornsby, 2007), the generation of a pro-tumourigenic environment via promotion of tissue damage such as MMP-dependent increases in the permeability of tumour capillaries (Liu and Hornsby, 2007), and the promotion of epithelial-mesenchymal transitions (EMT)(Canino *et al.*, 2012, Coppe *et al.*, 2008, Laberge *et al.*, 2012), a mechanism dependent on senescence-derived IL-6 and IL-8 *in vitro* (Coppe *et al.*, 2008), which can facilitate invasion and metastasis. Furthermore, due to its capacity to alter the microenvironment and promote inflammation (Hoenicke and Zender, 2012), the SASP has been proposed to contribute to tissue malfunction and degeneration.

These considerations raise two important questions. First, how can we rationalise these seemingly contradictory effects of the SASP? It is likely that the answer to this question lies within the nature of the SASP, which is highly context-dependent. Such a variable nature is likely responsible for the multiple, functionally antagonistic outcomes associated with cell senescence and suggest that a deep understanding of the specific senescence setting is required to determine its physiological impact. Second, given the wide range of detrimental effects associated with senescent cells, what burdens do they impose on an organism, and are there any silver linings to their functions?

A causal link between cellular senescence and ageing

Throughout their lifespan, mammals accumulate senescent cells in various vital organs and tissues including skin, heart, lung, liver, spleen and kidney (Wang *et al.*, 2009, Yang and Fogo, 2010). Whether this is due to decreases in functionality within the senescence clearance system, increases in the rate of senescent cell generation or loss of identification cues in senescent cells that may allow them to escape from immunesurveillance is not yet known (Burton and Krizhanovsky, 2014). Nevertheless, what is clear is that their accumulation is responsible for numerous decays in tissue structure and function that occur during Ageing. Compelling evidence from mouse models has recently established a casual link between the accumulation of senescent cells and a number of age-related disorders. In a seminal study by Van Deursen and co-workers, genetic elimination of $p16^+$ senescent cells in mice with a progeroid background caused by BubR1 deficiency was able to delay the onset of age-related disorders including cataracts, sarcopenia, osteoporosis and subcutaneous fat loss (Baker *et al.*, 2011), suggesting that cellular senescence is an important contributor to age-related decay. In this model, senescent cell elimination is achieved through expression of the INK-ATTAC transgene, which encodes a FKBP-Caspase8 fusion protein under the control of a senescence-responsive p16 promoter element (Baker *et al.*, 2016, Baker *et al.*, 2011). Upon treatment with the synthetic drug

AP20187, FKBP dimerisation leads to caspase activation and apoptosis induction in senescent cells (Baker *et al.*, 2016, Baker *et al.*, 2011). This transgene was subsequently used to study the effects of senescent cell elimination during normal Ageing in wild type mice (Baker *et al.*, 2016). Continuous drug treatment from one year of age onwards led to delays in tumourigenesis, attenuated age-related decay in several organs including kidney, heart and fat, and lead to significant (up to 30%) lifespan extension (Baker *et al.*, 2016). Moreover, recent evidence supports a direct role for cell senescence in the promotion of additional age-related disorders such as atherosclerosis (Childs *et al.*, 2016), allopecia (Yosef *et al.*, 2016), pulmonary fibrosis (Schafer *et al.*, 2017) and osteoarthritis (Jeon *et al.*, 2017). Together, these studies established senescence as an important cause of age-related organismal deterioration and led to the proposal that removal of senescent cells could prevent or delay tissue dysfunction and extend healthspan. This idea opened the door to the development of therapeutic strategies based on selective senescent cell elimination. These comprise the use of senolytics (as mentioned above), counteracting the effects of negative SASP components, and enhancing or engineering the immune system to promote senescent cell clearance. So far, senolytic treatment appears to be the most promising strategy, with several molecules that have been shown to decrease senescent cell populations *in vivo* without apparent side effects. These include the aforementioned apoptotic inhibitors ATB-263 (also known as Navitoclax) and ABT-737, UBX0101, and the peptide-mediated induction of p53-dependent apoptosis. In addition, a senolytic regime comprising treatment with dasatinib and quercetin has been shown to target certain types of senescent populations, though not all. A key issue with this (and other) strategies is the heterogenic nature of senescent cell populations. It is possible that, in practice, treatment may require the combination of several compounds or strategies. The two remaining approaches are as yet in their infancy, and will require the identification of critical mediators of specific negative functions of senescent cells (highly context-dependent), and the identification of specific senescent cell surface markers or 'eat me' signals that could be applied in the engineering of immune cells for improved senescent cell clearance.

Senescence in regenerative decays

Further extending its relevance to age-related deterioration, senescent progenitors or stem cells have also been shown to accumulate *in vivo* in aged tissues and in progeroid mouse models. Early studies had already suggested that they key senescence inducer p16 increased with age in regenerative progenitors. Notably, its overexpression contributes to the replicative failure of many regenerative cell types (Krishnamurthy *et al.*, 2006, Molofsky *et al.*, 2006), while its downregulation or genetic deletion ameliorates age-associated functional and proliferative impairments in stem and progenitor cells (Braun *et al.*, 2012, Janzen *et al.*, 2006), suggesting that cellular senescence could play a role in the decline in regenerative capacity with aging. More recently, direct evidence of such a role came from studies in murine muscle regeneration, which revealed that geriatric muscle stem cells (MuSC) lose their reversible quiescent state during aging by undergoing cellular senescence (Cosgrove *et al.*, 2014, Sousa-Victor *et al.*, 2014). This switch, which renders MuSC unable to activate and expand upon muscle injury, is triggered by an age-related increase in p38 pathway activation combined with the loss of polycomb repressive complex activity, resulting in increased p16 expression and senescence induction. Pharmacological inhibition of p38 or specific silencing of p16 in geriatric satellite cells restores both their reversible quiescence and regenerative functions (Cosgrove *et al.*, 2014, Sousa-Victor *et al.*, 2014). Furthermore, systemic treatment with the senolytic compound ABT-263 has also been shown to improve MuSC-based regeneration in ageing mice (Chang *et al.*, 2016, Yosef *et al.*, 2016). These beneficial effects of senescent cell elimination have also been observed in other systems in which regenerative capacity declines with age, including haematopoietic (Chang *et al.*, 2016), adipose (Xu *et al.*, 2015) and mesenchymal stem cells (MSC)(Li *et al.*, 2017). In the latter, it has recently been shown that the levels of the transcription factor FoxP1 decline during Ageing, leading to increases in p16 expression and MSC senescence. Conditional deletion of FoxP1 in mice promotes p16 derepression and MSC senescence resulting in decreased bone mass and loss of MSC self-renewal capacity (Li *et al.*, 2017). Lastly, a recent study reported a role for autophagy in maintaining MuSC quiescence by preventing senescence. Failure of autophagy in aged satellite cells, or genetic impairment of autophagy in young cells, causes entry into senescence by loss of proteostasis, resulting in functional and population declines in MuSC. Importantly, re-establishment of autophagy prevents senescence in MuSC, restoring their regenerative potential (Garcia-Prat *et al.*, 2016). Together, these studies suggest that maintenance of the quiescent state relies on the active repression of senescence pathways, and that cellular senescence, in a cell-autonomous manner, is a major contributor to the age-related decline in regenerative abilities.

Aside from its cell-autonomous role, cell senescence has been proposed to affect regenerative capacities through the SASP, by promoting tissue degeneration, niche alterations, progenitor malfunction or inflammation (Yun, 2015). In support of this idea, a negative role for senescent adipogenic progenitors has been described during ageing, whereby these cells secrete Activin A leading to inhibition of adipogenesis in non-senescent progenitors *in vivo* (Xu *et al.*, 2015). Furthermore, recent studies analysing the development of osteoarthritis have identified senescence as a negative driving factor of such pathology, which acts through the promotion of an anti-regenerative environment, dependent on MMP secretion, that affects cartilage development (Jeon *et al.*, 2017). Thus, besides its direct effects, senescent cells can promote regenerative declines indirectly, through their paracrine activities.

Shifting paradigms

Such a wide range of detrimental outcomes has recently ignited discussions on the evolutionary rationale for cellular senescence. While it constitutes a tumour-suppressor mechanism, the SASP can be tumour-promoting. Furthermore, the organism already possesses an alternative, and arguably more powerful, tumour-suppression mechanism: apoptosis. How then to explain the evolutionary persistence of cell senescence? One possibility is that the negative effects of cellular senescence are chiefly manifested during the post-reproductive period and thus avoid natural selection. Alternatively, senescent cells could play positive roles in physiological contexts. Indeed, in the past few years senescent cells have been found to contribute to several major processes including embryonic development (Munoz-Espin *et al.*, 2013, Storer *et al.*, 2013), pancreatic β-cell function (Helman *et al.*, 2016) and responses to tissue injury such as wound healing (Demaria *et*

al., 2014, Jun and Lau, 2010), fibrosis restriction following tissue injury (Krizhanovsky *et al.*, 2008, Meyer *et al.*, 2016) and tissue homeostasis (Ritschka *et al.*, 2017), highlighting the numerous beneficial facets of cellular senescence.

The silver linings of cellular senescence

Senescence in development

Senescent cells are found during restricted time-windows during the development of several structures in most vertebrates (Fig. 2), from amniotes (birds and mammals) to anamniotes (amphibian and fishes)(Chuprin *et al.*, 2013, Davaapil *et al.*, 2017, Munoz-Espin *et al.*, 2013, Nacher *et al.*, 2006, Storer *et al.*, 2013, Villiard *et al.*, 2017). Their developmental functions have been well characterised in structures such as the mouse and amphibian embryonic kidney, where they are part of a mechanism that promotes the degeneration of transient kidney forms (mammalian mesonephros and amphibian pronephros) to give rise to the ensuing, more mature kidney system (Davaapil *et al.*, 2017, Munoz-Espin *et al.*, 2013). Senescence is induced in pro- or mesonephric tubules at particular developmental stages, spreads through the structure with time, and leads to recruitment of monocytes/macrophages that results in senescent cell clearance and associated degeneration of the structure (Davaapil *et al.*, 2017, Munoz-Espin *et al.*, 2013). Cells within the apical ectodermal ridge (AER) of the mouse limb have also been shown to undergo senescence, which has been proposed to affect the underlying limb mesenchyme through paracrine cues that modulate growth and patterning. As in kidney development, these cells remain within the structure until their immune-mediated removal at later developmental stages (Storer *et al.*, 2013). To date, the collection of studies addressing their roles in various systems suggest that senescent cells contribute to tissue remodelling during development by three mechanisms: promotion of structural degeneration (mediated by macrophage-dependent elimination of senescent cells, exemplified by the embryonic kidney), balance of cell populations (senescence-mediated growth arrest of a particular cell population favouring growth of an alternate one, exemplified by the endolymphatic sac), and morphogenetic signalling (mediated by the SASP and controlled by immune-dependent clearance, exemplified by the mouse AER). In most cases analysed, the enforcement of cell cycle arrest is achieved through p21, and is independent of DNA-damage and p16, although p15 expression is detected in the mouse mesonephros and endolymphatic sac (Munoz-Espin *et al.*, 2013, Storer *et al.*, 2013), and p53 in the axolotl pronephros (Davaapil *et al.*, 2017). The signalling pathways involved in triggering developmental senescence are less conserved however. For example, TGFβ plays a key role in promoting senescence in the mouse mesonephros, salamander pronephros and *Xenopus* cement gland, while AER senescence relies on activation of ERK signalling in the underlying mesenchyme but is dispensable for pronephros generation. Distinct signalling could be associated with eliciting different types of senescence, which play equally different roles. Importantly, pharmacological inhibition of these signalling pathways or genetic disruption of p21 leads to loss of senescence accompanied by developmental abnormalities in various structures and organisms, suggesting that senescent cells do have a significant role in tissue remodelling during vertebrate development (Davaapil *et al.*, 2017, Munoz-Espin *et al.*, 2013, Storer *et al.*, 2013). However, it is noteworthy that in all cases analysed, functional defects are only observed transiently and the organisms survive without apparent developmental defects, due to the existence of compensatory mechanisms. These include apoptosis, which has been proposed to replace the functions of developmental senescence in absence of p21 (Munoz-Espin *et al.*, 2013, Storer *et al.*, 2013). Another interesting observation is that, while some functions of developmental senescence are conserved across vertebrates (such as embryonic kidney degeneration), others are not (such as the AER, which does not undergo senescence during amphibian limb development (Yun *et al.*, 2015)). Furthermore, senescence occurs during the development of structures that are not present in amniotes, such as the amphibian cement gland (Davaapil *et al.*, 2017). Together, these observations have several implications.

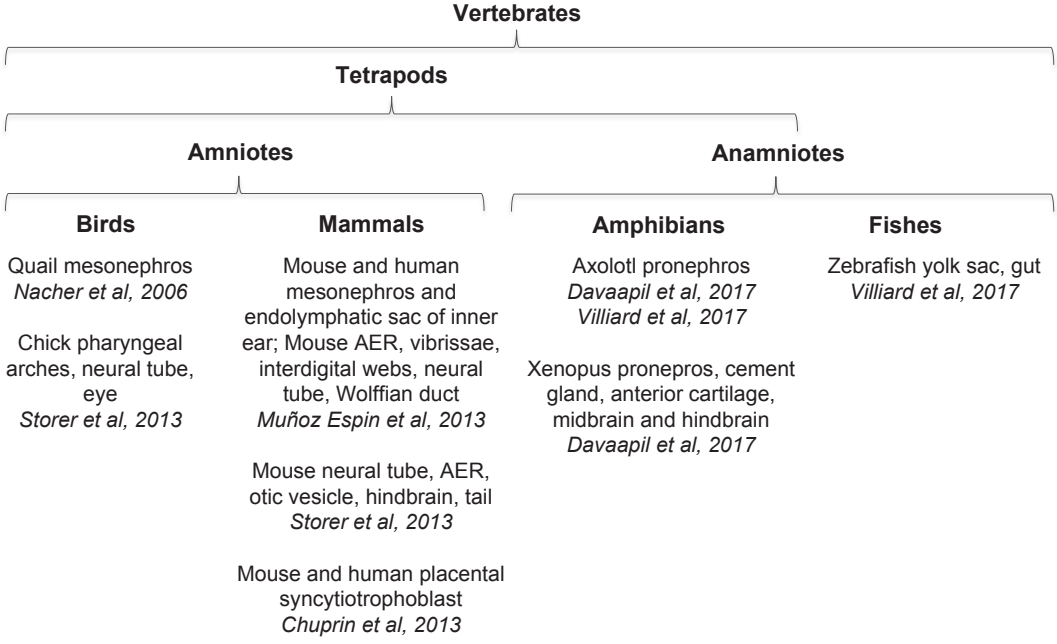

Fig. 2. Programmed cellular senescence is intrinsic to vertebrate development. *Senescent cells are found during limited time windows during the development of multiple structures across vertebrates, including in amniotes and anamniotes. These cells can contribute to tissue remodelling through the promotion of tissue degeneration, population balance or delivery of morphogenetic signals, as exemplified by the development of the embryonic kidney (mouse mesonephros and axolotl pronephros), the endolymphatic sac of the inner ear, and the mouse limb.*

TABLE 1

CELLULAR SENESCENCE IN PHYSIOLOGICAL CONTEXTS OF TISSUE INJURY

Wound healing	Tissue repair/homeostasis	Regeneration
Alleviation of fibrosis in skin by CCN1-mediated induction of myofibroblast senescence (*Jun and Lau, 2010*) In vivo acceleration of wound closure by senescent cells through induction of myofibroblast differentiation through secretion of PDGF-AA (*Demaria et al, 2014; Baker et a 2016*)	Senescence of activated stellate cells limits liver fibrosis (*Krizhanovsky et al, 2008*), mediated by CCN1 (*Kim et al, 2013)* CCN1-dependent senescence of myofibroblasts limits myocardial fibrosis (Meyer et al, 2016) Senescent cells are found during natural muscle repair in mouse (*Le Roux et al, 2015*) Exogenously-induced OIS favors skin homeostasis in vivo and promotes tissue-specific expression of stem cell markers in skin and liver cells (*Ritschka et al, 2017*); elimination of endogenous senescent cells promotes hair-follicle regeneration(Yosef et al, 2016)	Senescent cells are recurrently induced during salamander limb regeneration, coinciding with the generation of regenerative progenitors, followed by their rapid clearance (*Yun et al, 2015*) → Function?

Senescent cells are found in various types of regenerative processes. During wound healing, cellular senescence contributes to the acceleration of wound closure and the alleviation of fibrosis via non cell-autonomous mechanisms. In tissue repair and homeostasis, senescent cells play positive roles through cell-autonomous prevention of fibrosis and have been proposed to promote cell plasticity upon injury. In regeneration, senescent cells are recurrently induced coinciding with the generation of regenerative progenitors, yet their functions remain unknown.

First, they suggest that cellular senescence is a non-essential but intrinsic part of vertebrate development. Second, they insinuate that the functions of senescent cells arose early in evolutionary terms and in connection with developmental processes, perhaps predating other forms of senescence. Lastly, they suggest that cell senescence could have been incorporated in the developmental programme at various time points during vertebrate evolution.

An emerging player in responses to injury

Interestingly, in the past few years evidence has started to emerge suggesting the participation of cellular senescence in responses to tissue injury (Table 1). Such responses usually involve distinctive types of regenerative phenomena which, according to the current view of the field, can be divided into three major processes: wound healing, tissue repair and regeneration (Galliot *et al*., 2017). Each of these are characterised by particular kinetics, triggers, molecular and cellular requirements, and can occur with different outcomes in different organisms. As concisely summarised by Galliot *et al.,* wound healing entails the process of full or partial tissue restoration upon wounding and, in mammals, is often disrupted by fibrosis. Tissue repair refers to the restoration of an injured organ without exact patterning reconstruction, as in the case of liver, heart and muscle. In contrast, regeneration involves extensive regrowth and patterning of a complex structure, such as a part of an organ or a full appendage, and requires the generation and mobilisation of multiple cell type progenitors followed by their coordinated differentiation and spatial organisation (Galliot *et al*., 2017). To date, cellular senescence has been shown to contribute to wound healing and tissue repair. Furthermore, recent evidence has hinted at a potential role for senescence in regeneration of complex structures (Yun *et al*., 2015).

A critical aspect of wound healing is the maintenance of tissue integrity surrounding the wound, a process that relies on the deposition of extracellular matrix (ECM) and which should be tightly controlled, as it otherwise leads to fibrosis and scarring. Notably, cellular senescence is part of the mechanisms exerting such control, limiting fibrosis during wound healing (Jun and Lau, 2010). Following a phase of proliferation and ECM deposition, myofibroblasts at the wound site undergo senescence, which arrests their cell cycle and promotes expression of ECM degrading enzymes. Senescence is triggered by CCN1 (also known as CYR61), a matricellular protein dynamically expressed during wound healing. *In vitro*, CCN1 induces senescence through its interaction with integrins and heparan sulfate proteoglycans at the cell surface leading to the activation of NADPH oxidase 1 and ROS production, which then triggers p53 activation and p16 expression via the ERK and p38 MAPK pathways. *In vivo*, defects in CCN1 lead to lack of myofibroblast senescence at the wound site and exacerbate fibrosis, which can be reverted by topical application of purified CCN1, highlighting the importance of senescence as a fibrosis-limiting mechanism during wound healing (Jun and Lau, 2010).

The importance of senescence in fibrosis control is further supported by a more recent study, which uncovered additional functions of cell senescence in wound closure (Demaria *et al*., 2014). Using mice carrying a transgenic cassette for specific p16+ senescent cell labelling and elimination (p16-3MR), Demaria *et al.,* described that senescent fibroblast and endothelial cells are transiently induced at wound sites, where they accelerate wound closure. This is likely due to the senescence-mediated induction of myofibroblast differentiation, dependent on the secretion of platelet-growth factor AA (PDGF-AA) by senescent cells. Elimination of senescent cells leads to moderate delays in wound healing, a decrease in myofibroblast appearance (as estimated by the marker smooth muscle actin), and alterations in granular tissue resulting in fibrosis. Importantly, topical treatment with PDGF-AA is able to revert the alterations in wound closure kinetics and the decrease in myofibroblasts, while it does not revert the fibrotic increase, suggesting that the latter depends on other factors, such as MMPs, secreted by senescent cells (Demaria *et al*., 2014). Of note, wound closure is completed at 12 days both in the presence or absence of senescent cells, suggesting that this function of cell senescence, much like in development, is either non-essential or redundant. Together, these studies revealed that cell senescence has positive functions in wound healing contexts. While the effect of persistent endogenous senescent cells was not addressed in this work, it is of note that chronic, non-healing wounds have been shown to contain cells with senescent traits (Vande Berg *et al*., 2005). It is therefore possible that injury-induced senescent cells have beneficial effects when transient, yet detrimental ones when permanent, such as the promotion of inflammation, leading to wound healing impairments. This is a recurrent concept when considering the physiological roles of senescent cells, and one that merits further investigation.

The beneficial side of senescence is also evident in the context of tissue repair following liver or heart injury. In the liver, chronic damage results in fibrosis, which can eventually progress towards cirrhosis. In murine models of chronic liver injury, activated hepatic stellate cells undergo cell proliferation and ECM deposition, forming a fibrotic scar (Kong *et al*., 2012, Krizhanovsky *et al*., 2008). Mir-

roring the events during skin wound healing, these cells eventually undergo senescence and this limits their proliferation and promotes ECM degradation, thereby restricting the fibrotic response (Krizhanovsky *et al.*, 2008). This is followed by the immune-mediated clearance of these cells, achieved through natural killer (NK cells), thus completing the cycle (Krizhanovsky *et al.*, 2008). Mechanistically, hepatic stellate cell senescence is dependent on CCN1 for its induction, as seen in the skin. Interestingly, experiments using mice with hepatocyte-specific *Ccn1* deletion revealed that CCN1-induced senescent cells are not required for liver development or regeneration, but to inhibit fibrogenesis. Similar findings have been reported in contexts of myocardial fibrosis, where myofibroblasts were also found to undergo senescence dependent on CCN1 (Meyer *et al.*, 2016). Cardiac-specific expression of CCN1 results in reduction of perivascular fibrosis, whereas genetic ablation of p53 and p16, which disrupts the myofibroblast senescent programme, leads to increased heart fibrosis (Meyer *et al.*, 2016). Together, these findings establish CCN1-dependent cell senescence as a critical mechanism for the control of fibrosis.

A link between senescence and fibrosis is also found during muscle repair. In mice, inactivation of the endocytic adaptor Numb results in persistent p53-dependent senescence of myogenic cells following severe injury, leading to an *in vivo* decline in regenerative potential (Le Roux *et al.*, 2015). E*x vivo* experiments suggest that this decline could be explained by the generation of an inflammatory, pro-fibrotic environment caused by macrophage recruitment to senescent cells (Le Roux *et al.*, 2015). Thus, this provides further support for the recurrent concept that persistent senescent cells can have detrimental effects, such as the exacerbation of fibrosis. Interestingly, this study also described the induction of a transient, non-myogenic senescent cell population following muscle injury. While the functions of this population were not characterised, this finding raised questions about their nature and impact on tissue repair.

Recently, two studies offered interesting insights into such questions by uncovering another facet of cellular senescence: its effect on cellular reprogramming. Using a 'reprogrammable' mice strain (expressing the Yamanaka factors OCT4, SOX2, Klf4 and c-MYC (OSKM) in an inducible fashion) which enables *in vivo* reprogramming of adult cells into induced-pluripotent cells (Abad *et al.*, 2013), it was shown that various types of senescence, including senescence induced by bleomycin damage in the lung or snake venom cardiotoxin in the muscle, can enhance the efficiency of reprogramming in mice stimulated with OSKM (Chiche *et al.*, 2017, Mosteiro *et al.*, 2016). Efficiency of reprogramming is decreased in conditions which limit senescence, such as combined genetic deletion of p16 and ARF or treatment with the senolytic ABT-263, while conditions leading to the elevated presence of senescent cells (regardless of the type of senescence), such as palbociclib (a p16 functional mimetic) treatment, tissue damage, X-irradiation and Ageing (Chiche *et al.*, 2017, Mosteiro *et al.*, 2016) increase it. The effect of senescent cells is likely mediated by the SASP, as disruption of NFκb abrogates the positive effect on reprogramming (Mosteiro *et al.*, 2016). In particular, the secreted cytokine IL-6 has been proposed as a direct mediator of this effect, as treatment with anti-IL-6 antibodies decreases reprogramming efficiency while the opposite is observed upon recombinant IL-6 treatment *in vivo* (Chiche *et al.*, 2017, Mosteiro *et al.*, 2016), recapitulating prior observations *in vitro* (Brady *et al.*, 2013). Although the reprogrammable mouse is far from a normal *in vivo* system, these studies helped establish that cell senescence can be an inducer of cellular plasticity. This idea was also supported by a recent study showing that H*RasV12*-dependent oncogene-induced senescence, through the SASP, can lead to promotion of stem-cell markers in injury contexts such as skin and liver (Ritschka *et al.*, 2017). Ritschka *et al.,* showed that keratinocytes undergoing OIS acquire markers of somatic and cancer stem cells such as CD34, Lgr6 and Nestin, despite developing a senescent phenotype, both *in vitro* and *in vivo*. This depends on the SASP, as it is abrogated by NFκb inhibition. Furthermore, proliferating newborn primary mouse keratinocytes transiently exposed to OIS-derived condition media (2 days) *ex vivo* acquire skin stem cell markers and lead to increased hair follicle generation in a skin graft assay i*n vivo* (Ritschka *et al.*, 2017). Whether this represents an enhancement of stem-ness on keratinocytes that are not yet fully mature, or a *de novo* induction of stem-ness, needs further investigation. Yet, this work demonstrates that that OIS can stimulate cellular plasticity by promoting stem-ness. In addition, the authors went further and tested the effect of longer exposure to the OIS-derived SASP. In contrast to the effects after transient treatment, a 6-day exposure led to acquisition of senescence features in the mouse keratinocytes, which was suggested as an anti-tumourigenic response to counteract the promotion of stem-ness (Ritschka *et al.*, 2017), although whether this occurs in physiological conditions remains to be determined.

Together, these three studies show that senescent cells can lead to the promotion of two types of cellular plasticity, reprogramming and stem-ness. Whether this is a mechanism whereby endogenous senescent cells, induced in a normal physiological injury context, could promote regeneration (as proposed in the aforementioned studies) remains unknown. Thus, it is still unclear if this aspect of senescence is physiologically relevant in a natural repair context and what its implications are. While these studies put forward the hypothesis that senescence-mediated induction of plasticity could contribute to tissue repair, it has also been shown that elimination of endogenous senescent cells in various contexts is beneficial for repair processes. For example, senolytic treatment leads to an increase in hair-follicle stem cell proliferation (Yosef *et al.*, 2016), in disagreement with the aforementioned studies. Furthermore, senescent cells in Ageing contexts have been shown to promote cellular plasticity (Chiche *et al.*, 2017, Mosteiro *et al.*, 2016), yet they correlate with impaired regenerative responses to injury, which are improved upon senescent cell elimination (see previous section). Lastly, recent evidence from a model of Six1-induced senescence suggests that senescent cells can trigger a differentiation programme capable of limiting rather than promoting cellular plasticity (Adrados *et al.*, 2016). Although these disparate observations could perhaps be explained by contextual differences, they underscore that much is yet to be learned concerning the impact of senescence-induced plasticity. In addition, the promotion of highly plastic states such as those achieved through reprogramming is tightly associated with teratoma and tumour development (Abad *et al.*, 2013). This raises the possibility that the effect of senescence on cellular plasticity is another aspect of its pro-tumourigenic potential. Indeed, somatic cell reprogramming is emerging as a major process underlying generation of cancer stem cells (Friedmann-Morvinski and Verma, 2014). Therefore, defining to what extent endogenous senescent cells promote cellular plasticity, the type of plasticity they

elicit, whether this requires a particular microenvironment or cellular partners, and the mechanisms that regulate this function in *in vivo* contexts, would be essential for understanding their physiological roles, both in injury responses and elsewhere.

What about regeneration?

The finding that cellular senescence has positive roles in wound healing and tissue repair suggest the possibility that it could also play a role in natural regeneration. Although this suggestion has not yet been addressed directly, studies in the salamander limb regeneration model have recently delivered significant insights. Salamander limb regeneration is a striking example of regeneration that involves a rapid response to injury followed by the formation of a blastema (a mound of regenerative progenitors for the regeneration process), and the set up of a morphogenetic programme that allows the reconstitution of the limb to the original size and pattern specifications (Yun *et al.*, 2013). Notably, recent research (Yun *et al.*, 2015) found that senescent cells are recurrently induced during intermediate stages of regeneration, coinciding with the period of generation and expansion of regenerative progenitors, and are subsequently eliminated by a highly effective mechanism of senescence immunesurveillance which depends on macrophages (Fig. 3). These findings have several implications. First, they uncovered a highly efficient mechanism of immune surveillance operating in both normal and regenerating tissues that correlates with a lack of age-related accumulation of senescent cell in salamanders, the study of which could deliver new approaches for the elimination of senescent cells for therapeutic applications. Second, they provided the first evidence of senescent cell induction during regeneration, and opened the door to analysing whether transient induction of cellular senescence contributes to regeneration. Indeed, the reported dynamics of transient induction of senescent cells during key stages of regeneration followed by their timely elimination suggest that these cells could play positive roles in this process. A strong SASP signature has been reported in blastemas coinciding with peak induction of senescent cells (Yun *et al.*, 2015), raising the possibility that these cells have paracrine effects on the regenerate. Lastly, these findings have established the salamander as a model in which to study the effects of cellular senescence in natural regeneration. In this connection, it is of note that adult salamanders such as newts regenerate through the induction of a particular form of cellular plasticity, namely the tightly controlled dedifferentiation of mature differentiated tissues (Tanaka *et al.*, 2016), a regenerative mechanism whose preferential use could be responsible for their extreme resistance to tumourigenesis. Thus, they constitute a system in which to address the impact of senescent cells on dedifferentiation.

Integrating these observations with the current understanding of the functions of cell senescence in various contexts, it is possible to formulate a number of hypotheses for how senescence could contribute to regenerative responses (Fig. 4). The first one (a) consists of the direct promotion of regeneration via a SASP-mediated enhancement of the generation (though induction of dedifferentiation, stem-ness or other) or proliferation of regenerative progenitors, or the creation of a pro-regenerative tissue microenvironment (via ECM remodelling, metabolic reshaping, vascularisation). Alternatively (b), senescent cells could simply act by recruiting elements of the immune system, such as macrophages, T-cells and NK cells, which could then execute pro-regenerative functions. Components of the immune system have been shown to contribute to responses to injury in a wide range of contexts. In particular the macrophage, an essential cell type recruited to senescent cells, plays critical functions in wound healing (Lucas *et al.*, 2010), muscle regeneration (Ruffell *et al.*, 2009), neurogenesis (Kyritsis *et al.*, 2012) and regeneration of structures such as the salamander limb (Godwin *et al.*, 2013), the zebrafish fin (Petrie *et al.*, 2014) and the mouse digit tip (Simkin *et al.*, 2017), among others. Furthermore, phenotypic changes and even induced-senescence of the immune cells themselves following their recruitment should be considered. As mentioned, senescent cells can elicit changes in macrophage polarisation in certain contexts (Lujambio *et al.*, 2013), which can affect their functionality. Also, senescent NK cells have been shown to promote vascular remodelling and angiogenesis (Rajagopalan and Long, 2012), something that could be of relevance to their functions in injury responses. Lastly, a final possibility (c) is that cellular senescence could simply serve as a population balancing mechanism, as observed during the development of the endolymphatic sac of the inner ear (Storer *et al.*, 2013), leading to arrest and subsequent clearance of particular cells, controlling the proportions of certain populations versus others. These hypotheses consider the non cell-autonomous effects of senescent cells as mediated primarily by the molecules secreted to the environment. However, a recent study has shed light on an additional process, intercellular protein transfer, by which senescent cells can transfer molecular mediators to their neighbours via cytoplasmic bridges both *in vivo* and *in vitro*

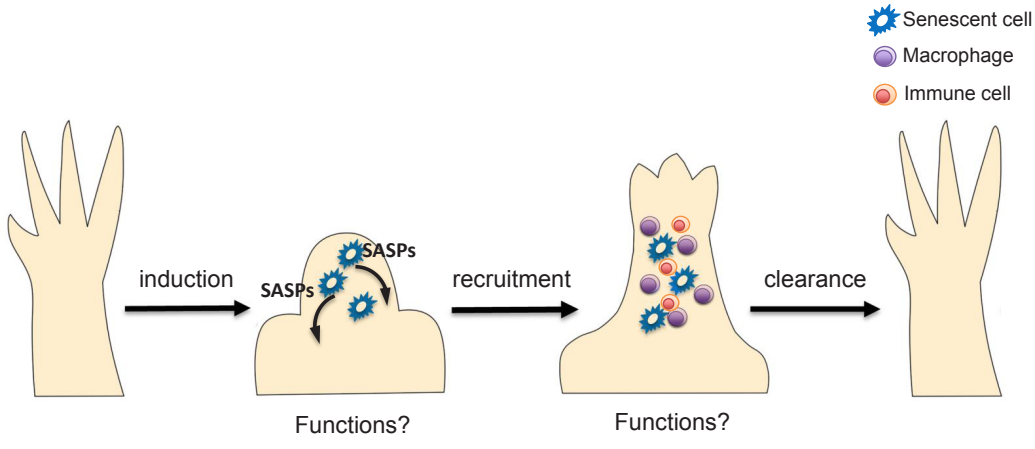

Fig. 3. Cell senescence in regeneration of complex structures. *Senescent cells are induced during regeneration and accumulate within the blastema. They produce a range of secreted molecules (SASP) which affect their microenvironment and could contribute, directly or indirectly, to different aspects of the regeneration process including matrix remodelling, vascularisation, cell plasticity, growth and patterning. Senescent cells are subsequently cleared by an efficient mechanism of macrophage-dependent immunesurveillance (Adapted from Yun* et al.*, 2015).*

(Biran *et al.*, 2015). This mechanism can mediate communication between senescent and epithelial and immune cells, and has been proposed to be relevant for senescent cell elimination (Biran *et al.*, 2015). It is possible that this process is also important in the context of regenerative responses, by enabling the promotion of cell-to-cell transfer of molecular mediators between senescent and regenerative or supporting cells.

An important component of these hypotheses is the timely elimination of senescent cells, as their persistence could be detrimental for the regenerative process (d). In pathological conditions or Ageing, senescent cells which persist through failures in clearance or increased/deregulated senescence induction rates leads to inflammation, niche disruption or, should it occur in progenitor cells, progenitor depletion (which explains the age-related decay seen in a number of systems), leading to regenerative impairments or tumour promotion. It is likely that the first two effects are mediated by the SASP. Through the course of a regenerative response, it is possible that the SASP evolves from a pro-regenerative to an anti-regenerative type; alternatively, perhaps the SASP does not change drastically but the functions of the SASP which could contribute to a regenerative process at one stage (e.g. promotion of cellular plasticity) become detrimental at a subsequent one. In both cases, senescent cell clearance would be required to avoid a pathological situation.

Together, these models highlight avenues for further research and at the same time underscore the current need for understanding the nature, role and regulation of cellular senescence in regenerative phenomena in the light of its potential biological and therapeutic importance.

Future perspectives

The impact of cellular senescence in physiological contexts, and in particular in responses to injury, is an exciting emerging area of research. As such, several important questions remain (Side Box). At the centre of these lies the molecular nature of senescent cells. Senescent cell populations are likely to be highly heterogeneous, with phenotypes that depend on the induction mode, cell type, time since induction and cellular interactions within the microenvironment. Given such heterogeneity, it is reasonable to ask if all cells

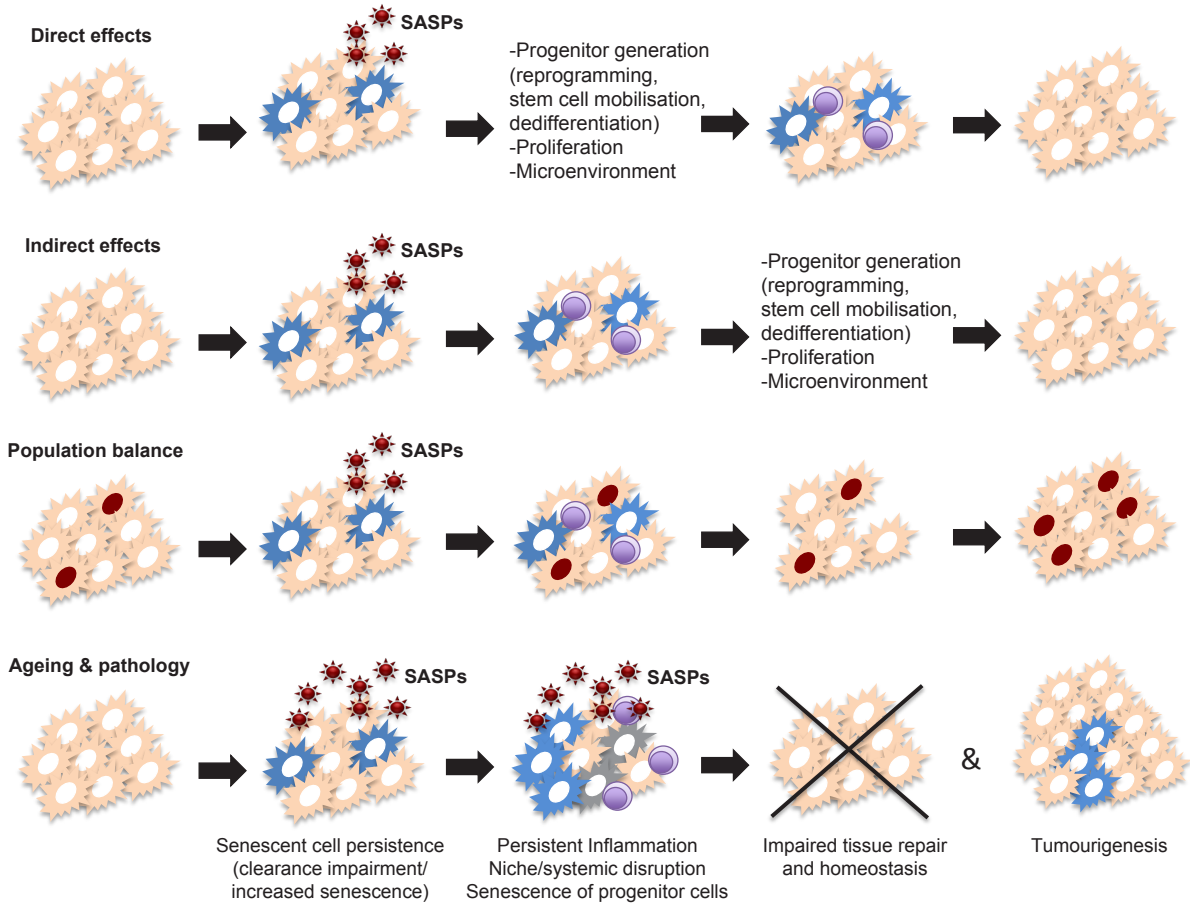

Fig. 4. Hypotheses for the functions of senescent cells in regenerative processes. *Transient senescent cells could promote tissue repair and regeneration directly (a) by enhancing the generation or proliferation of regenerative progenitors, or generating a pro-regenerative microenvironment through the SASP. Alternatively, they could exert these functions indirectly (b), via recruitment of immune cells which could themselves have pro-regenerative roles. Lastly, they could contribute to a population balancing mechanism (c), whereby senescence induction followed by clearance leads to the enhanced contribution of non-senescent cell populations to the final structure. Persistence of senescent cells and/or failure of senescence surveillance in ageing and/or pathological conditions (d), can lead to persistent inflammation, niche disruption or progenitor depletion, resulting in impaired regenerative responses and/or tumourigenesis.*

Side box - Outstanding questions

What is the level of heterogeneity -cell type composition, phenotype- among senescent cell populations?

To what extent does this impact on their phenotype and functions?

What are the common molecular denominators among different types of senescence?

How is senescence induced in physiological injury contexts – is it programmed and if so what are the triggers?

To what extent and by which mechanisms does cellular senescence impact diverse forms of cell plasticity? Is this pro-tumourigenic?

What is the effect of senescent cells in other cell populations *in vivo*?

Do senescent cells exert their functions directly or via engagement of other cell types (e.g. immune cells)?

How and by which mechanisms does the senescent phenotype evolve in time?

How and when does this phenotype become negative in injury contexts?

How is their elimination regulated? Are there general mechanisms or is it cell/context-dependent?

What are the effectors and signals mediating senescence clearance *in vivo*?

within a particular population could trigger a particular effect, or only a proportion. Furthermore, it is not yet clear how senescent cells are induced *in vivo*, how does the SASP evolves in time, if/when it acquires negative traits and how their clearance is regulated in physiological contexts. With regards to their effects on regenerative responses, the recent findings that cell senescence could contribute to the modulation of certain types of cell plasticity are exciting. However, it is not yet clear if this is an important mechanism by which endogenous senescent cells contribute to responses to tissue injury. There are different types of cell plasticity (pluripotency, stem-ness and controlled dedifferentiation) which are mechanistically very different processes, with particular requirements and equally diverse physiological effects. Thus, it would be important to determine to what extent senescent cells impact on cellular plasticity in various physiological contexts. Addressing these issues may underscore potential targets for therapeutic applications and offer a framework for the development of strategies for the treatment or prevention of pathological conditions.

With regards to therapeutic applications, the finding that senescent cells have beneficial functions in contexts of tissue injury is highly significant. First, the identification of molecular mediators of such functions, likely SASP-associated molecules, could provide therapeutic targets for pro-regenerative interventions. Second, in light of the recent development of senolytic strategies, recent findings will guide such approaches so that the negative effects of senescence (e.g. promotion of an age-related pathology) are suppressed, while maintaining the positive effects (e.g. wound healing or fibrosis limitation). This may imply the use of senolytic treatments conditioned by space and time or the design of combined therapies, accompanied by a thorough consideration of the physiological costs and benefits of every therapeutic intervention.

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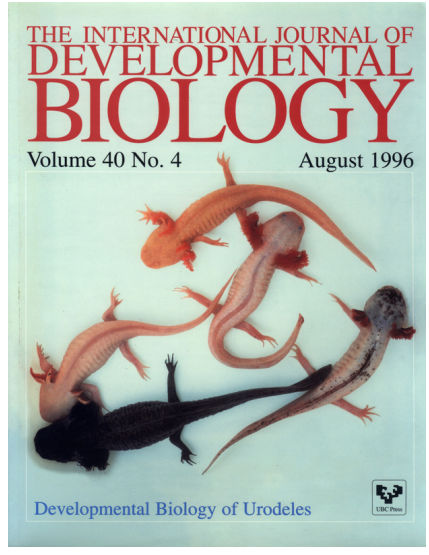
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