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PRIMER

Cellular senescence in development, regeneration and disease

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ABSTRACT

Cellular senescence is a state comprising an essentially irreversible proliferative arrest combined with phenotypic changes and pronounced secretory activity. Although senescence has long been linked with aging, recent studies have uncovered functional roles for senescence in embryonic development, regeneration and reprogramming, and have helped to advance our understanding of this process as a highly coordinated and programmed cellular state. In this Primer article, we summarize some of the key findings in the field and attempt to explain them in a simple model that reconciles the normal and pathological roles for senescence. We discuss how a primary role of cellular senescence is to contribute to normal development, cell plasticity and tissue repair, as a dynamic and tightly regulated cellular program. However, when this process is perturbed, the beneficial effects turn detrimental and can contribute to disease and aging.

KEY WORDS: Senescence, Embryo, Regeneration, Aging, Plasticity, SASP

Introduction

Cellular senescence is a form of permanent cell cycle arrest that can be induced in primary cells in response to a variety of stimuli. Senescence was first discovered in primary cells that were grown for extended periods in culture, reaching what became known as a state of replicative senescence, the cellular equivalent of old age (Hayflick, 1965). Subsequently, it was shown that cells exhibiting markers of senescence accumulate in aging tissues, further linking the senescence process with aging (Dimri et al., 1995). Later, a landmark study identified that the expression of active oncogenes (such as those encoding mutant Ras) in primary cells could induce senescence prematurely, in a process now known as 'oncogene-induced senescence' (OIS) (Serrano et al., 1997). This introduced the concept that senescence might function as a tumor-suppressive mechanism to block the aberrant proliferative effects of oncogenic mutations in cells. Following on from this, many diverse stress-inducing stimuli including irradiation (Le et al., 2010), chemotherapy (Schmitt et al., 2002), cytokine treatment (Braumüller et al., 2013) and even induced pluripotent stem cell (iPSC) reprogramming (Krizhanovsky and Lowe, 2009) have been shown to induce a senescent response in a variety of cell types. In summary, senescence functions as a cellular process that prevents the proliferation of old, damaged and potentially tumorigenic cells, but the consequence of which is increased aging at the organismal level.

However, more recent studies have uncovered beneficial effects of senescence, for example in the context of embryonic development, tissue repair/regeneration, and cellular reprogramming. As we review here, these discoveries have helped to broaden our understanding of the biological functions of the senescence process.

The senescence program

A primary feature of senescence, which separates it from quiescence or cell-cycle arrest, is a state of irreversible proliferative withdrawal. In tissue culture, senescent cells often exhibit a large flattened morphology, sometimes having multiple nuclei and large vacuoles. However, these size and shape changes may not occur in the same way in tissues. An additional feature of senescent cells is that they are resistant to apoptosis-inducing stimuli, a factor that likely contributes to their survival (Baar et al., 2017; Chang et al., 2016; Yosef et al., 2016). At the molecular level, the senescence program consists of two main components – the intrinsic arm and the extrinsic arm – that are broadly activated irrespective of the inductive stimulus, but exhibit some context-specific features, as discussed below (Fig. 1).

The intrinsic arm

The intrinsic arm regulates cell cycle arrest and is broadly mediated by key regulatory proteins including the p53 (also known as Trp53 or TP53), p21 (Cdkn1a), $p16^{INK4A}$ and $p19^{ARF}$ (both encoded by the *Cdkn2a* locus) tumor suppressors, which act to block the cell cycle and establish the irreversible arrested state (Kuilman et al., 2010; Martínez-Zamudio et al., 2017; Narita et al., 2003; Serrano et al., 1997). Senescence arrest is also fine-tuned by microRNA-mediated gene silencing (Benhamed et al., 2012). Some inducers of senescence also cause DNA damage, so immunostaining for markers of DNA damage such as γH2AX and 53BP1 can be used in some cases to identify senescent cells. However, it should be noted that no single marker can be used to identify all senescent cells (see Box 1).

Complex changes in 3D chromatin organization within the nucleus, as well as epigenetic changes, also occur in senescent cells. Changes in the nuclear lamina, including loss of lamin B1, occur in many states of senescence, and are suggested to enable spatial rearrangement of heterochromatin (Freund et al., 2012). In some cases of senescence, the formation of heterochromatin complexes known as senescence-associated heterochromatin foci (SAHF) is observed. These complexes consist of repressive chromatin regulators and marks, including HP1, MacroH2A, H3K9me3 and H3K27me3, concentrically layered to repress proliferation-associated genes and condense chromosomes. These epigenetic- and chromatin-mediated changes occurring in and regulating senescence have recently been reviewed in detail (Parry and Narita, 2016).

The extrinsic arm

The extrinsic arm of the senescence program consists of the 'senescence-associated secretory phenotype' (SASP). This is a

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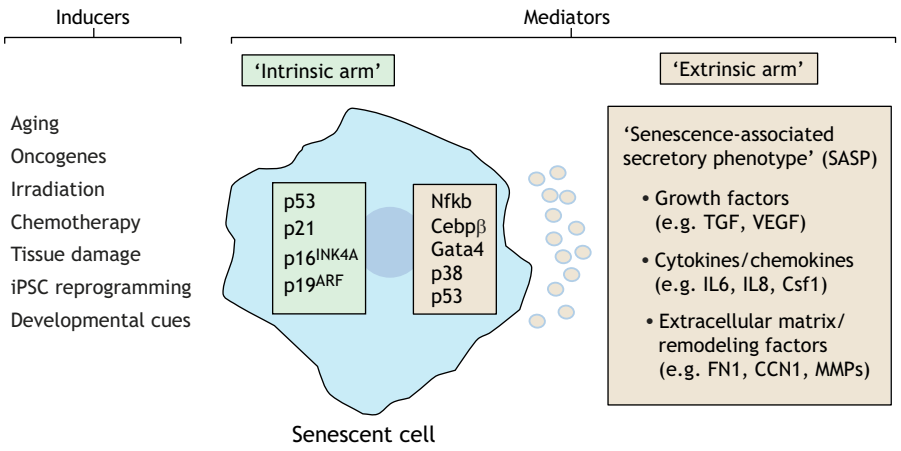

Fig. 1. Overview of the cellular senescence program. Senescence can be induced in response to a variety of inducers (left). Once activated, the senescence program then involves a number of key factors ('mediators') that mediate both the intrinsic and extrinsic arms of the senescent response. The intrinsic arm includes the tumor suppressor genes *p53*, *p21*, *p16*^{INK4A} and *p19*^{ARF}. These help to establish the cell cycle arrest and coordinate the complex senescence program. Furthermore, the activation of transcription and signaling factors including Nfkb, Cebpß, Gata4 and p38, in addition to p53, controls the extrinsic arm, a key part of which is known as the 'senescence-associated secretory phenotype' – the secretion of a cocktail of proteins by senescent cells that enables their interaction with the neighboring environment.

hallmark feature of senescent cells that reflects their ability, even though they are arrested from proliferation, to produce a rich secretome to interact with their external environment (Coppé et al., 2008). Although a detailed understanding of the composition of the SASP is still emerging, it is broadly composed of growth factors, cytokines, chemokines, and extracellular matrix (ECM) and ECM-remodeling proteins (Acosta et al., 2013; Coppé et al., 2010b; Freund et al., 2010) (Fig. 1). The regulation of this secretion is also highly coordinated and dynamic. Primary transcriptional regulators of the SASP include the Nfkb, Cebpβ, p53 and Gata4 transcription factors (Acosta et al., 2008; Kang et al., 2015; Kuilman et al., 2008), but also p38 MAPK, which regulates a DNA damage-independent SASP (Freund et al., 2011), and Notch1, which orchestrates a switch in SASP composition during senescence onset (Hoare et al., 2016). In addition, there is a pronounced epigenetic regulatory component to SASP control, with MLL1 (KMT2A), HMGB2, H2A.J and MacroH2A relocalization occurring early after senescence induction to regulate SASP gene expression (Aird et al., 2016; Capell et al., 2016; Chen et al., 2015; Contrepois et al., 2017). Although it is not yet known how SASP composition differs precisely in response to different stimuli, or between cell types, it is clear that the strength and mode of senescence induction is reflected in the SASP. For example, senescence induced by oncogenes such as the Ras genes, or following DNA damage, results in a more pronounced SASP than that induced by other factors (Coppé et al., 2008; Rodier et al., 2009).

Why senescent cells secrete such a rich cocktail of factors has been the subject of many studies. Initially, primary functions attributed to the SASP included reinforcement of cell cycle arrest by cytokines such as IL6 or IL8 via the CCR2 receptor (Acosta et al., 2008; Kuilman et al., 2008). Further, it was found that chronic exposure to the SASP can induce senescence in a paracrine manner in neighboring cells (Acosta et al., 2013). Functionally, some SASP proteins such as Csf1, Ccl2 and IL8 (Cxcl15) promote the recruitment of immune cells, including macrophages and natural killer (NK) cells, which remove senescent cells (Krizhanovsky et al., 2008; Lujambio et al., 2013; Xue et al., 2007). Such functions are in agreement with reinforcing the tumor suppressive role of senescence. Recently, additional cellular features of senescent cells and SASP regulation have been described, including the budding-off of chromatin fragments from senescent nuclei (Ivanov et al., 2013). Interestingly, these senescence-associated nuclear fragments are recognized by the anti-viral defense response, activating the cGAS-STING pathway, which contributes to SASP control (Dou et al., 2017; Gluck et al., 2017; Yang et al., 2017). However, additional effects of the SASP have also been discovered, such as the ability to induce proliferation, angiogenesis or epithelial-mesenchymal transition (EMT) in neighboring or cancer cells (Coppet al., 2010a, 2006; Gonzalez-Meljem et al., 2018; Krtolica et al., 2001). Together, these effects have suggested broader biological roles for senescent cells and the SASP, which are harder to reconcile with a simple tumor suppressive or aging function.

Senescence in disease and aging

Much of what we know about the role of senescence comes from *in vivo* genetic manipulation of either the cell-intrinsic or the cell-extrinsic aspects of senescence in different contexts of cancer and aging. In 2005, a series of studies demonstrated that oncogenic mutations in different contexts activate senescence *in vivo*, as had been previously shown in cells in culture, and that pre-malignant lesions, including papilloma or adenomas in the skin, lung, pancreas, lymphoma and prostate, form through an accumulation of senescent cells (Braig et al., 2005; Chen et al., 2005; Collado et al., 2005; Lazzerini Denchi et al., 2005; Michaloglou et al., 2005). Furthermore, these studies demonstrated that inactivation of the cell-intrinsic senescence machinery, through loss of function of key senescence genes such as *p53*, *p16*^{INK4A} or *p19*^{ARF}, prevents full senescence arrest and allows senescence bypass and malignant progression. These findings supported the notion that senescence is a tumor-suppressive barrier to cancer formation, and demonstrated how the most frequently mutated tumor suppressors in human cancers help protect from cancer by inducing senescence. In support of this, subsequent elegant studies have reported that the re-expression of p53 within p53-deficient solid tumors leads to reactivation of senescence in tumor cells (Xue et al., 2007). Interestingly here, the induced senescent tumor cells also activate an SASP and are actively removed by the immune system, showing how both the cell-intrinsic and cell-extrinsic arms of senescence can have tumor suppressive function (Lujambio et al., 2013; Xue et al., 2007).

However, although the regulated induction of senescence is beneficial in preventing tumor formation, prolonged aberrant persistence of senescent cells can have detrimental effects in promoting cancer. For example, if the timely clearance of OIS cells by the immune system is perturbed, this leads directly to tumor formation (Kang et al., 2011). Similarly, although chemotherapy can, in part, exert beneficial effects by inducing tumor-cell senescence (Schmitt et al., 2002), the persistence of therapy-induced senescent cells can, via the SASP, promote tumor recurrence and metastasis (Demaria et al., 2017; Zacarias-Fluck et al., 2015).

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Box. 1. Markers of senescence

One of the main challenges in senescence research is that there is currently no single marker that can be used to identify all senescent cells. The most commonly used is 'senescence-associated β-galactosidase' (SA-ß-gal), which makes use of the increased amount and activity of the enzyme β-galactosidase in enlarged lysosomes, which catalyzes a color reaction in cells at lower pH, turning them blue in the presence of X-gal (Dimri et al., 1995). This is analogous to the staining of *lacZ* reporter mice, but at a lower pH (5.5) and in the absence of a transgene, using instead the endogenous β-galactosidase gene *Glb1*. However, it is possible to have senescent cells that do not stain with SA-ß-gal, as demonstrated in cells lacking *Glb1* (Lee et al., 2006) and, for example, in mouse papilloma (Ritschka et al., 2017). Conversely, it is possible to have false-positive staining from macrophages (Hall et al., 2017). In addition, it appears that some tissues in the embryo stain positive for SA-ß-gal while not expressing other key markers such as p21 (Huang and Rivera-Pérez, 2014). Therefore, caution and diligence are needed when claiming senescence identification and, at a minimum, cells that are suggested to be senescent should exhibit a combination of senescence markers and features. Importantly, efforts are ongoing to identify potential new markers of senescence, including cell-surface proteins (Althubiti et al., 2014; Kim et al., 2017; Sagiv et al., 2016), commonly expressed senescence genes (Hernandez-Segura et al., 2017; Wiley et al., 2017) and histological stains (Evangelou et al., 2017).

The senescence process has long been linked to aging, including in the original study demonstrating that aging human skin has increased numbers of cells that are positive for the senescence marker senescence-associated beta-galactosidase (SA-ß-gal) (Dimri et al., 1995). In addition, the de-repression of senescence mediators including $p16^{INK4A}$ occurs during chronological aging, and contributes to loss of regenerative capacity in many tissues (Bracken et al., 2007; Krishnamurthy et al., 2006; Krishnamurthy et al., 2004; Sousa-Victor et al., 2014). In recent years, perhaps the most conclusive data linking senescence with organismal aging has come from the use of senescence 'deletor' mouse models, in which cells expressing $p16^{INK4A}$ are selectively targeted for elimination (Baker et al., 2016, 2011). In such models, the removal of senescent cells results in significant improvements in health and vigor, and also in lifespan. These studies unequivocally demonstrate how the accumulation of senescent cells during aging can have a negative impact on health and lifespan. Although these effects were primarily shown in response to targeting the cell-intrinsic program, it is likely that the SASP is also diminished in these models.

Similar studies using senescence-ablation mouse models have uncovered detrimental effects of senescent-cell accumulation in many other diseases, including osteoarthritis (Jeon et al., 2017), osteoporosis (Farr et al., 2017), atherosclerosis (Childs et al., 2016), Parkinson's (Chinta et al., 2018), Alzheimer's (Bussian et al., 2018; Musi et al., 2018) and others, whereas the selective deletion of $p16^{INK4A}$-positive cells improves many disease symptoms. The use of such models has spurred the search for new strategies to eliminate senescent cells, including drugs ('senolytics') and nanoparticles that eliminate senescent cells ('senotherapy'), which have been found to improve health or aging in many cases (Baar et al., 2017; Chang et al., 2016; Muñoz-Espín et al., 2018; Schafer et al., 2017; Xu et al., 2018; Yosef et al., 2016). In addition, new and previously known drugs are being investigated as SASP modulators for potential therapeutic uses, including glucocorticoids, metformin, Jak/Stat inhibitors and others (Soto-Gamez and Demaria, 2017), and possibly even small molecules targeting STING (also known as Tmem173) (Haag et al., 2018). Together, such approaches highlight how the accumulation of senescent cells can be detrimental to health and demonstrate the beneficial effects of senescent cell elimination or manipulation.

Roles for senescence in development, regeneration and reprogramming

As described above, much of what we understand about senescence has been extrapolated from studies of disease or aging. However, more recent discoveries of beneficial roles for senescence in non-disease conditions has helped to create a clearer understanding of the physiological function of senescence.

Wound repair

Beneficial roles for senescent cells have been described in various conditions of wound repair. After wounding, the deposition of ECM aids the repair process but, if excessive, can result in fibrosis, which subsequently impairs proper repair. Senescence has been demonstrated to have a role in wound repair and the fibrotic response in a number of tissues, including the liver (Krizhanovsky et al., 2008), skin (Jun and Lau, 2010), lung (Schafer et al., 2017) and heart (Zhu et al., 2013). In a mouse model of liver damage and fibrosis, senescent hepatic stellate cells were identified in the fibrotic lesions (Krizhanovsky et al., 2008). Interestingly, induction of damage in mice deficient for both the *p53* and $p16^{INK4A}$ genes, which exhibit an almost complete absence of senescence, results in increased fibrosis, suggesting that senescence limits the size of the fibrotic scar. Furthermore, the senescent cells in this context were shown to secrete SASP factors that promote the recruitment of immune cells, in particular NK cells, which subsequently eliminate senescent cells and the fibrosis. Thus, in response to tissue damage, senescence can arrest the proliferation of damaged cells, limit scar formation and program the removal of senescent cells by recruiting immune cells. However, in the absence of proper removal, an accumulation of senescent and fibrotic tissue occurs.

Indeed, the contribution of the senescent cells to the fibrotic lesion appears to depend in part on their duration and level of activation. For example, in the skin, the matricellular protein CCN1 becomes expressed following wound induction and activates a senescence response in fibroblasts, including DNA damage and p53 and $p16^{INK4A}$ expression (Jun and Lau, 2010). As in the liver, this coordinated response limits the fibrotic scar and contributes to the wound healing process. In skin wounds, the SASP also plays a role, whereby senescent fibroblasts and endothelial cells secrete an SASP containing PDGF-AA. Surprisingly, premature elimination of these transient senescent cells using a senescence-deletor mouse model results in impaired wound healing, demonstrating how senescent cells and the SASP are required for optimal healing (Demaria et al., 2014). However, in mouse models of idiopathic lung fibrosis induced by bleomycin treatment, senescent cells accumulate and contribute to the fibrotic disorder, whereas their elimination improves the condition (Schafer et al., 2017). Therefore, it seems likely that coordinated and timely production of senescent cells is beneficial in controlling the wound and early fibrotic response. However, if this becomes mis-regulated, senescent cell accumulation can have a negative impact on tissue repair.

It is not only mice that exhibit senescence following injury. The transient induction of senescent cells has been observed following limb amputation in the salamander; these cells are subsequently cleared by macrophages (Yun et al., 2015). Interestingly, the elimination of macrophages in this context results in persistent senescence and impaired regeneration, further supporting the idea that timely removal of senescent cells is needed for tissue repair and regeneration. Interestingly, salamanders appear not to accumulate

senescent cells with additional damage, as these cells are consistently cleared even upon repeated amputation (Yun et al., 2015).

Developmental senescence

The discovery of cells exhibiting markers and features of senescence in developing embryos was an exciting finding. This was primarily based on studies describing senescent cells in mouse embryos (Muñoz-Espin et al., 2013; Storer et al., 2013). However, cells bearing some or many features of senescence have also been described in human (Muñoz-Espin et al., 2013), chicken (Storer et al., 2013; Gibaja et al., 2019), quail (Nacher et al., 2006), *Xenopus* (Davaapil et al., 2017), axolotl (Davaapil et al., 2017; Villiard et al., 2017), zebrafish (Villiard et al., 2017) and naked mole rat (Zhao et al., 2018) embryos (Table 1).

In the mouse, the incidence and distribution of senescence was initially described between embryonic day 9.5 and 15.5 (Muñoz-Espin et al., 2013; Storer et al., 2013). Staining of embryos with the senescence marker SA-ß-gal identified many tissues containing senescent cells, including the apical ectodermal ridge (AER) of the developing limb, the hindbrain roofplate, the mesonephros, the neural tube, the endolymphatic sac, the pharyngeal arches, the tip of the tail and the gut endoderm (Table 1). Interestingly, in many cases, senescent cells were found in signaling centres, with the secretory function of these structures contributing to cell fate specification and tissue patterning.

Other studies similarly described cells bearing markers of senescence in a variety of organisms, including *Xenopus*, axolotl and zebrafish, in tissues including the pronephros, olfactory epithelium, nerve fascicles, yolk sac, midbrain and hindbrain (Table 1) (Davaapil et al., 2017; Nacher et al., 2006; Villiard et al., 2017; Zhao et al., 2018). Furthermore, senescent cells have been described in the extra-embryonic tissues of the placenta in mouse and human, where cell-cell fusion between maternal and fetal cells leads to senescence in the resulting syncytiotrophoblasts (Chuprin et al., 2013).

Initially identified using SA-ß-gal, cells in the mouse were deemed senescent based on their expression of multiple markers including SA-ß-gal, p21 and SASP factors, but also according to their proliferative arrest and ultimate clearance by macrophages. Interestingly, these cells were shown to be negative for other senescence markers including p53, $p16^{INK4A}$, $p19^{ARF}$ and markers of DNA damage, suggesting that senescence in the embryo might represent a different or simpler type of senescence. In addition, although p21-deficient animals are, for the most part, developmentally normal, it was shown that they have mild patterning defects in the limbs, kidneys and vagina (Muñoz-Espin et al., 2013; Storer et al., 2013). Furthermore, interference with the senescence program by chemical means (using TGFß or ERK inhibition), and more recently by senolytic treatment, leads to patterning defects (Muñoz-Espin et al., 2013; Storer et al., 2013; Davaapil et al., 2017; Gibaja et al., 2019).

Overall, the emerging details suggest that senescent cells may have multiple functions in the embryo. Senescent cells that appear in the embryo arise in very precise patterns in time and space, appearing during specific time windows, before subsequently disappearing, demonstrating that the induction, presence and removal of these cells is a tightly controlled programmed cellular process. Moreover, it should be emphasized that this occurs identically in every embryo, and the cells do not display markers of damage, demonstrating that this is not a stochastic damage response activated to eliminate some damaged cells, but rather that this is a normal programmed developmental process, under the control of highly organized instruction processes. Given their secretory nature, it appears that these cells contribute to the fine-tuning of cell fate specification and tissue patterning. However, these cells are subsequently removed, demonstrating a remodeling effect of their clearance on tissue patterning and removal of transient structures. In such a way, senescent cells act as a complementary process to apoptosis, which is also known to be important for development (Fuchs and Steller, 2011), and may even share common regulatory signals (Lorda-Diez et al., 2015).

Plasticity and reprogramming

Senescence is also intricately linked with cellular reprogramming, with studies of iPSCs providing key clues. Indeed, expression of the four reprogramming factors Oct4 (Pou5f1), Sox2, Klf4 and Myc (OSKM) causes widespread induction of senescence markers in cells that ultimately do not undergo reprogramming, whereas those that successfully reprogram manage to silence key senescence mediators. In addition, elimination of the senescence genes *p53*, $p16^{INK4A}$, $p19^{ARF}$ or *p21* significantly increases the efficiency of reprogramming, demonstrating that cell-intrinsic senescence is a barrier to reprogramming (Banito et al., 2009; Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009; Marion et al., 2009; Utikal et al., 2009).

Recent *in vivo* reprogramming studies have further increased our understanding of this connection. Interestingly, induction of reprogramming in tissues also activates a senescence response, but in cells adjacent to those that undergo reprogramming (Mosteiro et al., 2016). It appears that the SASP, and in particular IL6 from the senescent cells, enhances OSKM activity and reprogramming in nearby cells. Similarly, activation of the reprogramming factors in a muscle damage environment has demonstrated that damage- and age-induced senescence also favors the reprogramming of muscle satellite cells via the SASP (Chiche et al., 2017; Mosteiro et al., 2016). However, it was also shown that cyclic short-term expression of the reprogramming factors *in vivo* does not induce senescence, but instead promotes regeneration and improves aging (Ocampo et al., 2016).

It is not only in the context of reprogramming that senescence exerts beneficial effects on cell plasticity and regeneration. A recent study using oncogene- and irradiation-induced senescence in primary

Table 1. A list of organisms and their associated tissues in which senescent cells have been described during embryonic development

Species	Location of senescent cells	Reference(s)
Mouse	Apical ectodermal ridge (AER), hindbrain roofplate, mesonephros, endolymphatic sac, pharyngeal arches, gut endoderm, neural tube, tip of tail, placental syncytiotrophoblasts	Muñoz-Espin et al., 2013; Storer et al., 2013; Chuprin et al., 2013
Human	Mesonephros, endolymphatic sac	Muñoz-Espin et al., 2013
Chick	Pharyngeal arches, neural tube, AER, eye, otic pore, endolymphatic duct	Storer et al., 2013; Gibaja et al., 2019
Quail	Mesonephros	Nacher et al., 2006
Zebrafish	Yolk sac, gut	Villiard et al., 2017
Axolotl	Pronephros, olfactory epithelium nerve fascicles, lateral organs, gums	Davaapil et al., 2017; Villiard et al., 2017
Xenopus	Cement gland, midbrain, hindbrain, pronephros	Davaapil et al., 2017
Naked mole rat	Nail bed, skin (dermis, hair follicle), bone marrow	Zhao et al., 2018

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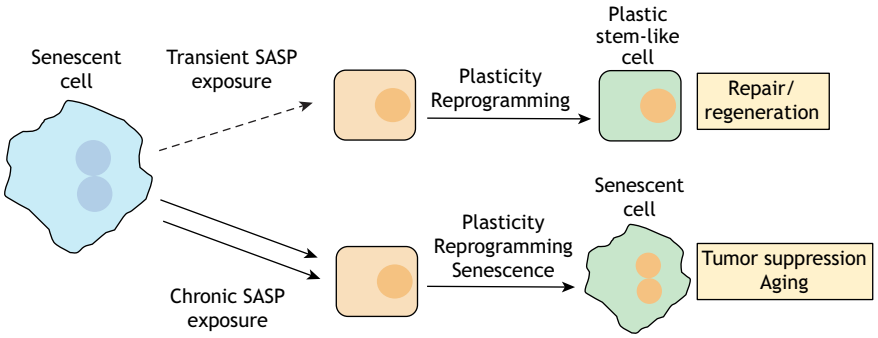

Fig. 2. Senescence and reprogramming. Summary of recent findings describing roles for senescent cells in promoting plasticity and reprogramming. It has been described that transient exposure to the SASP promotes plasticity and increases iPSC reprogramming capacity, ultimately favoring tissue repair and regeneration. However, chronic exposure to the SASP, although also sufficient to induce markers of plasticity and reprogramming, activates a cell-intrinsic senescence block to aberrant stemness and, ultimately, can contribute to tumor suppression and/or aging.

mouse keratinocytes showed that the SASP can induce a skin stem cell fate (Ritschka et al., 2017). Remarkably, primary mouse keratinocytes transiently exposed to the SASP undergo dedifferentiation to become functional hair follicle stem cells that can regenerate the skin when grafted into mice. However, prolonged exposure to the SASP, although further increasing stem cell gene expression, subsequently activates cell-intrinsic senescence arrest and results in papilloma formation *in vivo* (Ritschka et al., 2017). More recently, two studies have identified how transient senescence contributes to heart regeneration, whereas elimination of senescent cells blocks proper heart regeneration (Feng et al., 2019; Sarig et al., 2019).

Together, these studies suggest that a key role of the SASP is to alter the plasticity of neighboring cells (Fig. 2). Transient exposure to the SASP increases the efficiency of reprogramming factors, and also favors plasticity and regeneration in tissues such as the skin, liver, muscle and heart (Chiche et al., 2017; Feng et al., 2019; Mosteiro et al., 2016; Ritschka et al., 2017; Sarig et al., 2019). However, if exposure to the SASP is prolonged, as shown in the context of the skin (Ritschka et al., 2017), then the resulting increased plasticity is likely sensed by target cells as abnormal or tumorigenic, and is subsequently blocked by the activation of cell-intrinsic barrier mechanisms. In a similar way, intrinsic senescence blocks reprogramming, and only those cells that can evade senescence can become iPSCs.

Reconciling the varied functions of senescence

Although the roles for cellular senescence in tissue development and reprogramming may initially appear to be different from those in cancer and aging, it is quite straightforward to reconcile their disparate functions. Below, we attempt to simplify and integrate our understanding of senescence biology into a simple scheme, based on the premise that transient and controlled induction of senescent cells is the desired scenario, and that the accumulation of senescent cells in aging and disease involves an aberrant mis-regulation of this program (Fig. 3).

Senescence in a physiological setting

Initiation

In the various settings described above, the senescence program is initiated, leading to commitment to cell cycle arrest and SASP establishment. In the embryo, this does not appear to involve DNA damage but instead is instructed by reciprocal signaling between secreted factors including TGFβ, and is mediated by p21. In adult tissue, tumor-suppressive senescence induction appears more complex, involving additional tumor suppressor genes including $p16^{INK4A}$, $p19^{ARF}$ and *p53*.

SASP

The arrested cell then secretes a cocktail of factors including ECM-modifiers, growth factors and cytokines that together function to instruct tissue repair, fibrosis, patterning and immune-cell recruitment. It is very probable that this secretion is controlled over time to adapt to the status of the tissue, while also acting to provide optimal growth conditions and to recruit the immune system in advance to where it will be needed. In addition, the SASP may play a role in macrophage polarization (Lujambio et al., 2013).

Patterning and plasticity

In some ways, it appears that senescent cells can function as a local regenerative niche or signaling center. The SASP induces functional changes in neighboring cells, including patterning and plasticity, as well as proliferation, angiogenesis or EMT. During limb development, the gradient of factors secreted from the AER (in combination with posterior signals) establishes plasticity in the limb mesenchyme (Cooper et al., 2011; Rosello-Diez et al., 2011). It appears that a similar function might be partially reactivated when senescence is induced postnatally, using oncogenes, irradiation or iPSC reprogramming (Mosteiro et al., 2016; Ritschka et al., 2017), or during heart regeneration (Feng et al., 2019; Sarig et al., 2019).

Clearance

The recruited immune cells (macrophages, NK cells) now contribute to the clearance of senescent cells and cellular debris. Whether the senescent cells undergo apoptosis before clearance, or are first removed by macrophages, during development remains to be shown. However, in the AER, as p21 protects senescent cells from apoptosis, the downregulation of p21 probably favors initial cell death before removal (Vasey et al., 2011). In the context of oncogene- or damage-induced senescence, by contrast, it is likely that there is little or no apoptosis, as p21 levels are maintained (Yosef et al., 2017), but senescent cells are cleared by the immune system within approximately two weeks (Kang et al., 2011; Xue et al., 2007). It also appears that salamanders have an efficient capacity to remove senescent cells even upon repeated wounding (Yun et al., 2015).

Outcome: development/regeneration

Ultimately, it appears that senescent cells need to be removed during development/regeneration. In situations of stress and/or damage, this would ideally eliminate the initial source of the damage and SASP, whereas in the embryo, this is probably achieved when the phase of instruction of developmental senescence has passed. In each case, the outcome is the development of the mature tissue form, or restoration of the pre-damaged state.

Senescence in a pathological setting

Initiation

It is very likely that similar inducers can activate the senescence program in old or damaged tissue, but the rate of induction of senescence is possibly increased owing to increased levels of damage

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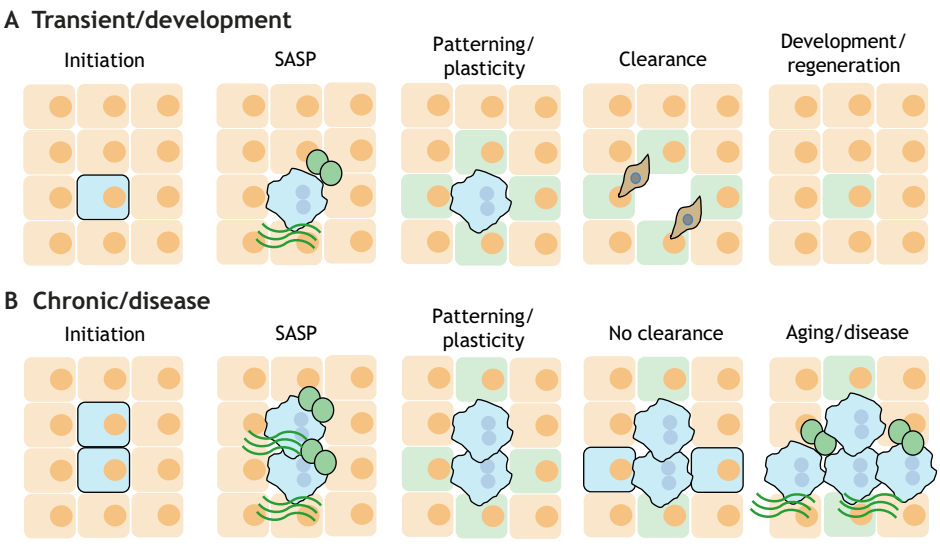

Fig. 3. Physiological and aberrant senescence. (A,B) Summary of the key features of senescent cells comparing transient (A) with chronic (B) senescence. In response to an inducer, a cell initiates the senescence program (blue). This cell activates the secretion of SASP factors such as cytokines and ECM factors (green circles and lines). One effect of the SASP is to favor plasticity in neighboring cells, while also recruiting immune cells such as macrophages (brown cells) to clear the senescent cell. This restores the pre-damaged state, or favors tissue development. In situations of chronic or disease states, an increased incidence of senescence results in exaggerated features of the program, including prolonged SASP, enhanced plasticity and accumulation of senescent cells, thus further enhancing tissue dysfunction and damage, and resulting in aging/disease.

in the cells, or inductive signals accumulated with age. In addition, the clearance of senescence is probably diminished, resulting in an amplifying loop of senescence–paracrine senescence instruction.

SASP

Because of the increased number of senescent cells, the SASP is also likely to be amplified or altered. For example, increased or prolonged secretion of ECM-modifying factors could alter tissue structure, contributing to the age-associated decreases in tissue renewal and maintenance, and increased fibrosis.

Patterning and plasticity

The functions of the SASP in inducing plasticity or dedifferentiation are also retained in aging, as increased reprogramming is seen when the OSKM factors are induced in an aged tissue (Mosteiro et al., 2016). It is interesting to speculate that such an induction of aberrant plasticity by senescent cells in their neighbors could lead to increased populations of tumor-initiating stem cells at risk of transformation (Gonzalez-Meljem et al., 2017).

No clearance

For unknown reasons, whether it is because there is an increased production of senescent cells, a dysfunctional immune system or altered recognition, senescent cells are not sufficiently cleared in aged tissue. This leads to an increased incidence of senescent cells and increased SASP from these cells.

Outcome: aging/disease

Ultimately, the accumulation of non-proliferating senescent cells directly impairs tissue proliferation, contributing to disease and, in situations of advanced age, directly blocking stem cell function (Sousa-Victor et al., 2014). In addition, the accumulated SASP from these cells likely continues to influence neighboring cells in an increasingly negative manner. Indeed, age-associated inflammation and SASP-factor accumulation can impact directly on stem cell function, which may be amenable to alleviation with anti-inflammatory SASP-modulator drugs such as Jak/Stat inhibitors (Doles et al., 2012). As such, the recent interest in genetic and drug strategies to eliminate such aberrantly accumulating senescent cells is already proving to have beneficial effects on lifespan, healthspan and disease severity (Baar et al., 2017; Chang et al., 2016; Muñoz-Espín et al., 2018; Schafer et al., 2017; Xu et al., 2018; Yosef et al., 2016).

Conclusions and future directions

During recent years, cellular senescence has emerged from being considered a cell culture artefact by some, to being understood as a highly complex and dynamic cellular program with diverse roles across the lifespan of an organism. The controlled induction of senescence appears to be beneficial in many conditions including tumor suppression, development, reprogramming and regeneration. However, as with many cellular processes, its mis-regulation can be detrimental. Future studies are clearly needed to unravel how, when and why this mis-regulation occurs. Obviously, there are many avenues to explore in senescence biology (see Box 2).

Perhaps one of the most intriguing questions is why senescent cells accumulate during aging. As of yet, we still do not explicitly understand why there are more senescent cells in aged tissues, however this likely arises through a variety of causes. For example, the locus encoding the $p16^{INK4A}$ and $p19^{ARF}$ genes loses repressive epigenetic marks during aging, which increases the sensitivity of these genes to be induced (Bracken et al., 2007; Martin et al., 2014). In addition, an accumulated SASP and tissue inflammation in aged tissue likely increases the induction of senescence, and spreads the response (Acosta et al., 2013; Xu et al., 2018), and a decline in phagocytosis during aging might also contribute to impaired removal of senescent cells (Li, 2013). Unraveling the mechanisms behind this aberrant accumulation will undoubtedly aid the therapeutic development and use of senescent cell manipulation. In this sense, perhaps a better understanding of the efficient clearance mechanisms that enable complete removal of senescent cells in the embryo, and whether/how these mechanisms operate in aged states, could shed some light.

Regarding developmental senescence, there are many unanswered questions. For example, how can the population of senescent cells arising during development inform us more generally about the program of senescence? Are these cells in the embryo fully senescent? Or do they represent a simpler precursor state of their age-associated counterpart? Or are these populations of cells in the embryo that express a set of markers of diverse cellular functions and that, for some reason, are reactivated upon damage or aging in adult tissues? One of the common features of developmental and adult onset senescence is the high level of p21 and SA-ß-gal, but neither alone is indicative of senescence. In the embryo, there are p21-positive cells, such as those in the midbrain-hindbrain boundary (Trokovic et al., 2005), that do not stain with SA-ß-gal, and many

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Box. 2. Outstanding questions

- What are the main similarities and differences between developmental senescence and oncogene-induced or aging-associated senescence?
- What are the cues that regulate developmental senescence?
- What are the consequences of mis-regulated senescence during development?
- Are the SASP components the same from different cell types and stimuli, how do they change over time (i.e. in early versus late senescence), and how can we distinguish SASP factors from tissue damage signals?
- Why do senescent cells accumulate during aging?

p21-positive cells may resume proliferation upon downregulation of p21 – for example, some p21-positive cells in the limb reenter the cell cycle (Li et al., 2018). However, as fate mapping of the mature AER demonstrates, this transient structure is removed (Guo et al., 2003), suggesting heterogeneity in the p21-expressing population within the limb. In addition, there are SA-ß-gal-positive cells that are not p21 positive, such as those in the visceral endoderm (Huang and Rivera-Pérez, 2014). One explanation is that it is likely that the level of p21 expression, in combination with other factors, determines the irreversibly arrested state. Another more complex explanation is that there are as yet unexplained differences between cells expressing senescence markers in the embryo and those that reactivate these markers in aging or disease, and that only direct detailed comparisons will uncover these differences.

It is interesting that many sites of developmental senescence are signaling centers. Although most of the studies to date have focused on mid-late stage embryos, it will be interesting to see whether senescence plays a role in earlier stages of development and/or organizer function, or whether it could be adapted as an additional quality control process to regulate an embryonic response to damage. Given that age- and damage-associated senescent cells are highly secretory, it is tempting to speculate that senescence-inducing stimuli in these settings may be reactivating developmental pathways of plasticity and patterning instruction. However, in adult settings, the recipient cells receiving such signals may not be as plastic as those in the embryo. Indeed, tumor suppressor genes such as $p16^{INK4A}$ and $p19^{ARF}$, which are silenced in the embryo, are increasingly re-expressed during aging and act to suppress regeneration (Krishnamurthy et al., 2004). Interestingly, these same genes are not expressed in animals that can regenerate, and their regulated induction is sufficient to block regeneration, as seen in the zebrafish tail and the axolotl spinal cord (Hesse et al., 2015; Khattak et al., 2013). Therefore, what we understand as damage-associated senescence may actually be a combination of beneficial developmental-like senescence properties, which are subsequently blocked by tumor-suppressive senescence with age. Of course, it is likely not as straightforward and simple as this, as it also appears that $p16^{INK4A}$ expression (and not that of $p19^{ARF}$) contributes in some cases to the beneficial effects of senescence and the SASP, such as reprogramming, wound repair and insulin secretion, whereas p53-independent p21 expression blocks reprograming (Demaria et al., 2014; Helman et al., 2016; Mosteiro et al., 2016, 2018). Only a thorough comparison of senescent cells in each setting will help to understand their true biological significance. Elucidating the detailed dynamics of the senescence program at a single cell level will help resolve these questions, as will future studies using specific markers/reporters and lineage tracing studies.

Another area that requires further study is the SASP. Although it is well documented that senescent cells secrete a vast array of factors, it is equally known that many such secreted factors are activated upon tissue damage and can induce senescence. Therefore, additional work is needed to unravel the dynamics of SASP control and content, and to identify which factors represent the initial damage response and which are true SASP factors. Such studies will obviously also benefit from single cell analyses in appropriate models, including the embryo.

The current interest in identifying senolytic drugs to delay aging, improve healthspan or alleviate disease symptoms is an exciting prospect, and the possibility of finding drugs that could aid in preventing the general decline associated with the aging process is almost inconceivable. However, recent studies suggest this may be a real possibility, and the first clinical trials have begun. Of course, such drugs will have to be rigorously tested, not only for beneficial effects of senescence elimination, but also to uncover any possible downsides. As senescent cells are increasingly shown to have beneficial functions, such as aiding in regeneration or limiting fibrosis, interference with these processes may hinder tissue repair or regeneration or ultimately even aid in tumor formation or recurrence. It will be important to identify context-specific senolytics for maximum efficiency.

An irreversible cell cycle arrest is a hallmark feature used in the definition of senescence. In response to therapy, cancer cells can also undergo senescence-like arrest. However, as the intrinsic tumor-suppressive mediators of senescence may be inactivated in cancer cells, some reports suggest that individual cancer cells might ‘escape’ from chemotherapy-induced senescence and drive tumor recurrence. Interestingly, the cells that escape may have increased cancer stem cell properties. Although some reports suggest this is an intrinsic dedifferentiation process, others suggest that SASP factors such as IL6 and thrombospondin 1 induce plasticity and stemness (Achuthan et al., 2011; Guillon et al., 2019; Milanovic et al., 2018; Zacarias-Fluck et al., 2015). Nevertheless, the induction and persistence of senescent cells following therapy may actually promote tumor recurrence, possibly mimicking their roles in development and regeneration and, as a result, there is increasing interest in strategies to eliminate them, including using senolytics as a secondary treatment (Demaria et al., 2017; Sieben et al., 2018).

Finally, it is worth mentioning that the link between senescence and congenital defects remains underexplored. Given that developmental senescence is found in some of the tissues that are most susceptible to mutation and birth defects, it is tempting to speculate that mis-regulation of senescence may be causally involved. Indeed, a number of studies in mouse models, including mice deficient in *p63* (Keyes et al., 2005), *PASG* (*Hells*; Sun et al., 2004) and *Brca1* (Cao et al., 2003), have shown how mutations that affect embryonic development also induce aberrant senescence, whereas recent studies using senolytics demonstrate how mis-regulation of the normal developmental process can cause patterning defects (Gibaja et al., 2019). A better appreciation of this link will further our understanding of senescence and its role in human health and disease. It is no doubt an exciting time for senescence research.

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Competing interests
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