Chapter 3
Interconnection Between Cellular Senescence, Regeneration and Ageing in Salamanders

Qinghao Yu and Maximina H. Yun

Abstract Urodele amphibians have long served as key models for regenerative, developmental and evolutionary biology research. Recent studies have uncovered the induction of cellular senescence during limb regeneration. The dynamics of senescence in this context reflects that observed in acute senescence, suggesting that senescent cells may play positive roles in regeneration. Further, salamanders possess a highly robust and efficient mechanism for senescent cell surveillance and clearance. Given the causal role of chronic senescence in ageing and age-related pathologies, it is of therapeutic interest to understand the mechanisms and regulation underlying this clearance mechanism. Here, we discuss what is known about cellular senescence in salamanders, what these organisms can offer towards understanding the roles of cellular senescence in regeneration, and how they can serve as informative models for senescence-based therapeutic approaches.

Keywords Cellular senescence · Axolotl · Newt · Regeneration · Development · Senolytics

3.1 Introduction

3.1.1 Cellular Senescence

Senescence is a stress response to severe genotoxic or cellular insults, in which cells enter a state of essentially irreversible growth arrest and acquire a set of characteristic phenotypic alterations (Hayflick and Moorhead 1961; van Deursen 2014; Campisi 2013). A variety of cell intrinsic and extrinsic stresses can trigger the senescence

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D. Muñoz-Espin and M. Demaria (eds.), *Senolytics in Disease, Ageing and Longevity*, Healthy Ageing and Longevity 11,
https://doi.org/10.1007/978-3-030-44903-2_3

response, such as telomere attrition (Bodnar et al. 1998), DNA damage (Sedelnikova et al. 2004; Di Micco et al. 2006), oxidative damage (von Zglinicki 2002), and chronic mitogenic signalling (Moiseeva et al. 2006). These senescence-inducing stimuli engage various cellular signalling networks, but ultimately implement the characteristic growth arrest by inhibition of cyclin-Cdk complexes through the activation of the tumour suppressors p53, p16, or both (Ben-Porath and Weinberg 2005). Both pathways converge at the level of Rb hypo-phosphorylation, resulting in the continued repression of E2F-target genes required for cell-cycle progression (Stein et al. 1990; Narita et al. 2003). In addition to the senescence-associated growth arrest, senescent cells exhibit a range of distinctive phenotypic alterations, including changes in cell morphology, chromatin remodelling (Shah et al. 2013; Narita et al. 2006; Zhang et al. 2007), metabolic reprogramming (Kaplon et al. 2013; Dorr et al. 2013), and an expansion of the lysosomal and mitochondrial networks. One of the most prominent features exhibited by the majority of senescent cells is the upregulation of genes that encode secreted proteins—an array of proinflammatory cytokines and chemokines, together with various growth factors and proteases—collectively referred to as the senescence-associated secretory phenotype [SASP] (Acosta et al. 2013; Coppe et al. 2010). This phenotype may vary depending on cell type, stressor and context. The SASP is a key distinguishing feature of senescent cells from other non-proliferating states, such as quiescence and terminal differentiation, and underlies many of the physiological and pathological functions of cellular senescence by facilitating communication with the surrounding tissue microenvironment.

The irreversible cell-cycle arrest implemented during replicative senescence was recognised early on as a powerful cell-autonomous mechanism to restrict the expansion of damaged cells (Hayflick and Moorhead 1961; Bodnar et al. 1998). The subsequent discovery of oncogene-induced senescence [OIS] reinforced the notion that cellular senescence, like apoptosis, constitutes a safeguard against tumorigenesis (Serrano et al. 1997; Michaloglou et al. 2005; Chen et al. 2005; Collado et al. 2005; Braig et al. 2005). In contrast to apoptosis, however, senescent cells remain viable and metabolically active and, as such, are able to influence tissue structure and function. Methods to identify and perturb senescent cells in vivo have extended its known roles beyond tumour suppression to a wide range of biological processes. Research in the past decades has placed cellular senescence as an integral component of embryonic development (Czarkwiani and Yun 2018; Munoz-Espin et al. 2013; Davaapil et al. 2017; Storer et al. 2013), wound healing (Jun and Lau 2010; Demaria et al. 2014), and tissue repair (Krizhanovsky et al. 2008; Kim et al. 2013; Meyer et al. 2016), highlighting positive roles for cellular senescence. The process can, however, exert deleterious effects, as illustrated by its causal role in ageing, age-related loss of regenerative capacity, and neoplastic progression (van Deursen 2014; Campisi 2013; Coppe et al. 2010; Krtolica et al. 2001; Laberge et al. 2012; Munoz-Espin and Serrano 2014). The disparate physiological effects of senescence are likely reflected by diversity on the cellular level, in terms of triggering stress, the kinetics and mechanism of induction, tissue context, and a corresponding heterogeneity in SASP composition. Thus, a more nuanced understanding of the physiological

Fig. 3.1 Urodele amphibians. **a** The commonly used d/d axolotl strain [left] and wild-type [right] adult axolotls [*A. mexicanum*]. **b** Post-metamorphic Iberian ribbed newts [*P. waltl*]

roles of cellular senescence has emerged: a current hypothesis holds that, in addition to acting as a cell-autonomous mechanism of tumour suppression, senescent cells function primarily to restore tissue homeostasis in response to acute damage and stress (Yun 2018). Subsets of cells within the damaged tissue enter the senescent state, and, likely through various components of the SASP, coordinate responses in the surrounding microenvironment [which may range from modulation of cellular plasticity to pro-regenerative ECM remodelling and vascularisation] to restore tissue and organ function. This response, referred to as 'acute senescence', culminates with the recruitment of components of the immune system that mediate the elimination of senescent cells. Among the critical findings contributing to this hypothesis are those obtained using unconventional yet rapidly developing model organisms, the salamanders [Urodele amphibians; Fig. 3.1] (Yun et al. 2015).

3.1.2 Salamanders as model organisms for senescence studies

Urodeles such as the Mexican axolotl [*Ambystoma mexicanum*] and Iberian ribbed newt [*Pleurodeles waltl*] possess a remarkable capacity for regeneration. A salamander can restore its limbs and tail, upper and lower jaws, ocular tissues such as the lens and retina, the intestine and portions of the heart and brain (Brockes and Kumar 2005; Brockes 1997). Following limb amputation, the amputation plane is sealed by the wound epidermis, a transient epithelium formed by rapid migration of epithelial cells from the wound circumference (Brockes and Kumar 2005; Brockes 1997). Regeneration then proceeds through the formation of a blastema, a transient mass of progenitor cells that proliferate and differentiate to restore the missing structure with remarkable fidelity and morphogenic autonomy (Brockes and Kumar 2005;

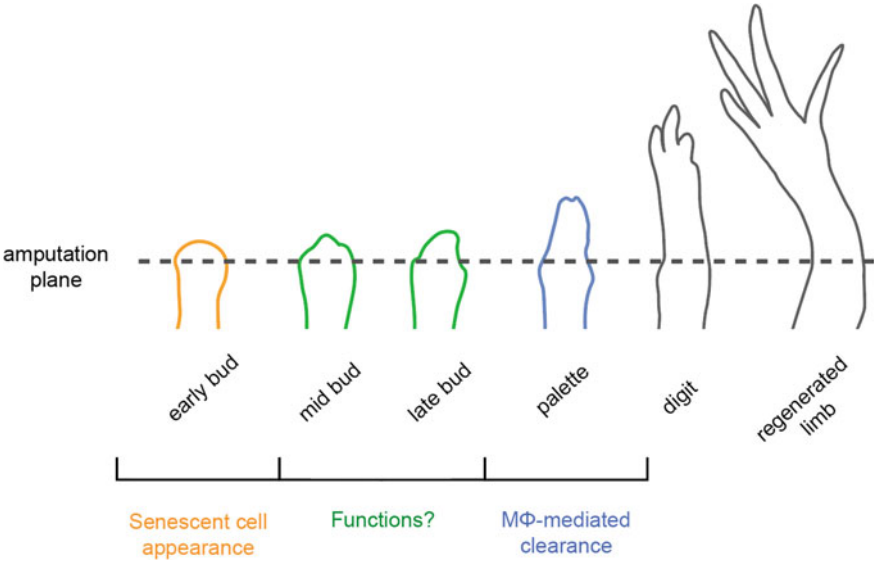

Fig. 3.2 The events and kinetics of senescent cells during salamander limb regeneration. Senescent cells appear during the early to intermediate stages of regeneration and accumulate in the blastema. They produce an array of secreted factors that can affect neighbouring cells, which could directly or indirectly contribute to different aspects of the regenerative programme

Tanaka 2016; Nacu and Tanaka 2011). It was recently reported that limb regeneration is accompanied by induction of a senescent population within the blastema, a process which is specific to regeneration and absent in limb development, and occurs recurrently with multiple rounds of regeneration (Yun et al. 2015). The events and kinetics of cellular senescence in this context are highly reminiscent of those observed during acute senescence, and proceeds with a timely induction and concludes with macrophage-dependent elimination (Fig. 3.2). These observations raise the intriguing possibility that senescent cells may be functionally important for regeneration of complex structures, such as—but not limited to—the salamander limb. Indeed, various lines of research have placed cellular senescence as an integral component in the response to injury, and highlighted its capacity for promoting tissue remodelling and maintenance (Yun 2018). The mechanisms by which senescent cells exert these effects may also be pertinent to urodele regeneration, and will be discussed in depth below.

Beyond the role of senescence in regeneration, salamanders also offer a model of relevance to senescence in ageing contexts. In contrast to the temporally-controlled induction of senescence during processes such as wound healing and embryonic development, the accumulation of senescent cells during organismal ageing appears to be stochastic and unscheduled, and likely occurs due to a combination of different stresses [chronic senescence] and a failure in immune-mediated clearance (van Deursen 2014; Wang et al. 2011). The increased prevalence of senescent cells with

age represents a driving factor in tissue deterioration and age-related pathologies (van Deursen 2014). Notably, salamanders do not appear to accumulate senescent cells with age, possess long lifespans without any obvious signs of tissue deterioration, and retain the capacity for repetitive rounds of regeneration throughout the entirety of their lifespans (Yun 2015). Thus, these organisms offer an interesting tool for comparative approaches towards understanding mechanisms that limit age-related senescent cell accumulation. In this review, we examine the interplay between cellular senescence, regeneration, and organismal ageing in salamanders. We discuss how insights from cellular senescence in other processes can inform us about the contribution of senescence towards the remarkable features of salamanders, and highlight what these organisms can offer as models to investigate the physiological roles of senescence.

3.2 Physiological Roles of Cellular Senescence

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3.2.1.1 Senescence in Wound Healing and Tissue Repair

Research into the functional roles of cellular senescence in different physiological contexts have illustrated that senescent cells hold tremendous potential in influencing tissue organisation and architecture. Through paracrine signalling mediated by the SASP, senescent cells can impact various biological processes, including cell proliferation, differentiation, angiogenesis, cellular plasticity, inflammation, and immunemodulation (Rajagopalan and Long 2012; Lujambio et al. 2013). The potential of senescent cells to coordinate tissue remodelling in vivo has been appreciated in the context of embryonic development, wound healing and tissue repair. In mammals, the response to tissue and organ damage often proceeds through fibrosis instead of regeneration. Excessive fibrosis can lead to scar formation and tissue dysfunction, and the failure to regenerate imposes a major clinical burden (Gurtner et al. 2008). Research over the past decade has revealed that cellular senescence is a central component of the response to tissue injury and damage, and plays an important role in limiting excessive fibrosis at injury sites through the production of SASP factors that promote matrix degradation (Yun 2018). Senescence-mediated restriction of fibrosis is observed in various systems, including the liver (Kim et al. 2013; Borkham-Kamphorst et al. 2014), skin (Jun and Lau 2010; Demaria et al. 2014), and heart (Meyer et al. 2016) and constitutes a conserved response during tissue repair and wound healing.

The extracellular matrix protein CCN1 is upregulated at injury sites and coordinates multiple aspects of wound healing (Kim et al. 2018). Through genetic and biochemical analyses, CCN1 has been found to promote tissue repair in numerous contexts, including skin injury and liver damage, by stimulating senescence in cells

at sites of injury (Jun and Lau 2010; Kim et al. 2013). CCN1 acts through integrin a6ß1 and HSPS-mediated activation of the RAC1-dependent NADPH1 oxidase, resulting in sustained reactive oxygen species [ROS] accumulation and consequent p53 and p16 activation (Jun and Lau 2010). Recombinant CCN1 is able to induce senescence in fibroblasts in vitro, and stimulate the expression of matrix degrading enzymes [MMP1, MMP3], pro-inflammatory cytokines, and significantly downregulate type I collagen expression. Indeed, when the integrin interaction of wild-type CCN1 is impaired in vivo, wound healing following skin injury proceeds with exacerbated fibrosis due to a failure of myofibroblasts to undergo senescence. Treatment of wounds with recombinant CCN1 in this background was able to limit excessive fibrosis and reduce collagen deposition. In the case of liver injury, CCN1 is produced by damaged hepatocytes, and is required for senescence induction in activated HSCs. Accordingly, mice with hepatocyte-specific deletions of CCN1 exhibit exacerbated liver fibrosis in response to damage (Kim et al. 2013; Borkham-Kamphorst et al. 2014).

A more recent study from Campisi and co-workers further examined the role of the secretome in the pro-regenerative effects of senescence during cutaneous wounding. Using a mouse model that enables inducible elimination of senescent cells, the authors showed that senescent cell depletion resulted in poor formation of granulation tissue at the wound site and reduced angiogenesis (Demaria et al. 2014). Characterisation of the senescent population revealed that these cells largely comprise fibroblasts and endothelial cells, and show elevated expression of the SASP components PDGF-A and VEGF. Senescent cell-depletion significantly delayed the kinetics of wound closure, a process which depends on the induction of contractile myofibroblasts. Histological analysis revealed a reduction in the number of myofibroblasts in the mid-region of wound sites, suggesting a potential decrease in myofibroblast differentiation. This idea was supported by the capacity of PDGF-AA to stimulate the differentiation of fibroblasts to myofibroblasts in vitro. Indeed, topical treatment of senescence-free wounds with recombinant PDGF-AA restored normal kinetics of wound closure, with concomitant restoration of myofibroblast numbers (Demaria et al. 2014). It should be noted that wound healing under these conditions still concluded with excessive fibrosis, consistent with additional components of the SASP being required for full wound resolution (Demaria et al. 2014).

The aforementioned studies support the idea that transient induction of senescent cells can promote certain types of wound healing. However, further research suggest that the timely elimination of those cells is equally important for the outcome of the process. Particularly, studies of the senescence response during liver damage have emphasised the importance of senescent cell clearance in tissue repair contexts (Krizhanovsky et al. 2008). Gene expression analysis of senescent HSCs revealed up-regulation of pathways involved in immune surveillance, including stimulating receptors for natural killer cell function such as MICA, ULBP2 and PVR2. Upon abrogation of NK cell-mediated clearance, senescent cells accumulated and treated livers displayed significantly more fibrosis as compared with controls (Krizhanovsky et al. 2008). Clearly, cellular senescence has an important role in fibrosis restriction in response to injury, and may also represent an important factor in the response to

amputation through regeneration rather than fibrotic scarring in salamanders (Yun 2018).

3.2.1.2 Developmental Senescence

Surveys for senescent markers in axolotl, *Xenopus*, mice, chick and quail embryos has revealed that senescence induction occurs consistently in discrete time windows in numerous structures during development (Munoz-Espin et al. 2013; Davaapil et al. 2017; Storer et al. 2013; Villiard et al. 2017). The major function of cellular senescence during development is to promote the regression of transient embryonic structures, through the recruitment of immune components. For example, senescence is induced in the tubules of the amphibian pronephros and the mammalian mesonephros [precursor embryonic kidney forms], spreads throughout the structure, culminating in monocyte/macrophage recruitment and subsequent senescent cell clearance and associated structural degeneration (Munoz-Espin et al. 2013; Davaapil et al. 2017). In addition, senescent cells are thought to modulate tissue patterning and morphogenesis, as exemplified by patterning defects in the cement gland and the oral and nasal cavities when TGF-ß signalling is perturbed in *Xenopus* embryos. In the murine apical epidermal ridge [AER], a key signalling centre during limb development, SASP-derived FGF8 and FGF4 serve as key proliferation-inducing signals to the adjacent mesenchyme, and loss of cellular senescence in p21 null embryos results in reduced proliferation in the underlying stroma and disrupts the normal expression of key patterning genes (Storer et al. 2013). Further, cellular senescence has been proposed to modulate the differential arrest and expansion of different cell populations. In the endolymphatic sac epithelium of the inner ear, senescence induction occurs in a subset of epithelial cells, and coincides with a robust expansion of a minor, pendrin-positive population beginning at E14.5 (Kim and Wangemann 2011). During murine development, the loss of p21-dependent senescence results in abnormal expansion of pendrin-negative cells, and the robust in pendrin-positive population is notably reduced, resulting in aberrant infoldings of the epithelium into the lumen (Munoz-Espin et al. 2013). In many cases analysed, developmental abnormalities are corrected at later stages through compensatory mechanisms such as apoptosis and late macrophage infiltration. In contrast to damage-induced senescence, growth arrest during development appears to be implemented mainly through p21, and occurs in the absence of DNA damage, although p15 is detected in the mouse mesonephros and endolymphatic sac, and p53 in the axolotl pronephros. The signalling networks underlying developmental senescence induction, however, appear to be less conserved. For example, TGFB plays a key role in senescence induction in the mouse mesonephros, salamander pronephros and the *Xenopus* cement gland (Davaapil et al. 2017). However, whilst ERK signalling is required for senescence induction in the mouse AER, ERK inhibition has no discernible effects on amphibian senescence (Davaapil et al. 2017).

These observations have opened several important questions. First, they demonstrate that cellular senescence is an intrinsic component of vertebrate development,

and can occur in a programmed manner in response to developmental cues outside of pathology. They also raise the question as to whether senescence initially evolved to orchestrate tissue remodeling and morphogenesis during embryonic development, perhaps predating other forms of senescence, and was co-opted for its roles in adult life later in evolution. Further, the species-to-species variation between developmental senescence [such as its presence and absence during mouse and amphibian limb development, respectively] and its involvement in clade-specific structures [such as the mammalian Wolffian duct and the amphibian cement gland] suggest that developmental senescence arose multiple times independently during vertebrate evolution (Czarkwiani and Yun 2018). The contributions of cellular senescence to tissue patterning and remodeling in these developmental contexts could also be of relevance to regeneration in salamanders, a process which requires careful control of patterning and coordination of multiple cell types in order to reconstitute a functional structure (Yun 2018).

3.2.1.3 The Interplay Between Cellular Senescence and Plasticity

Recently, an interesting interplay between senescence and the control of cellular plasticity has come to light. The ectopic expression of the transcription factors OCT4, SOX2, KLF4, and cMYC [OSKM factors] in vivo leads to reprogramming of adult cells into induced pluripotent stem cells [iPSCs] and the formation of teratomas [tumours derived from iPSCs] (Abad et al. 2013). Using a murine model for in vivo reprogramming, Serrano and colleagues uncovered a strong correlation between pluripotency induction and cellular senescence (Abad et al. 2013; Mosteiro et al. 2016). OSKM expression led to reprogramming in a subset of cells, and senescence in many others in close proximity. Using genetic and pharmaceutical analysis, it was found that abrogation of senescence through deletion of p16 or the senolytic agent navitoclax [ABT-263] severely compromised the efficiency of reprogramming in vivo. In contrast, under conditions whereby senescence is elevated, such as upon damage, in tissues of progeroid mice, or even in naturally-aged mice, reprogramming and teratoma formation increased. This effect is dependent on the SASP and, in particular, IL-6 was identified as a critical factor in mediating the interplay between cellular senescence and reprogramming (Mosteiro et al. 2016). In support of this, using a transplant model, Keyes and colleagues showed that exposure of primary keratinocytes to OIS-derived SASP enhanced the expression of stem-cell markers and increased their proliferative capacity after grafting (Ritschka et al. 2017). However, prolonged exposure to OIS-derived medium led to the acquisition of senescent traits and a loss of proliferative capacity, reemphasising the importance of the transient nature of senescence to its beneficial effects (Ritschka et al. 2017).

Although these studies have illustrated the potential for cellular senescence to impact cellular plasticity, whether the same mechanism applies to normal in vivo contexts is not yet clear. However, salamander models may shed light into this important question. Post-metamorphic salamanders such as *Notophthalmus viridescens* rely

on the tightly-controlled dedifferentiation of adult tissues to form regenerative progenitors (Brockes and Kumar 2005). Two informative systems have been used to analyse reversal of the differentiated state in urodeles: lens and limb regeneration in adult newts. Early work from Eguchi and co-workers showed that following lens removal, pigmented epithelial cells [PECs] of the dorsal iris re-enter the cell cycle, lose their pigmentation, and transdifferentiate into lens cells (Eguchi and Shingai 1971; Eguchi et al. 1974; Del and Tsonis 2003). In the case of muscle, regeneration proceeds through dedifferentiation of muscle fibres to generate proliferative mononucleate progenitors (Echeverri et al. 2001; Kumar et al. 2000; Kumar et al. 2004; Lo et al. 1993). The implantation of purified myofibres from culture or of iris tissue fragments into a blastema leads to the generation of mononucleate cells or lens, respectively (Lo et al. 1993; Reyer et al. 1973). The blastema thus provides an environment capable of destabilising the differentiated state without fully erasing cell identity and of promoting return to the cell cycle (Brockes 1998); whether this property is a function of cellular senescence is currently unknown. However, the advent of pharmaceutical and genetic approaches to perturb the blastemal senescent population (Box 3.1) in vivo positions salamander regeneration as a model to assess the interplay between naturally-occurring cellular senescence and plasticity. Indeed, the induction of senescent cells in the limb blastema coincides with the period of progenitor generation and expansion (Yun et al. 2015) and is consistent with a potential role in modulating cellular plasticity.

The enhancement of cellular plasticity through dedifferentiation and increased ‘stemness’ in the blastema raises the issue of tumorigenesis in urodeles. The early stages of regeneration share many parallels with tumorigenesis; yet, experimental evidence has shown that salamanders possess not an increased susceptibility, but rather a remarkable resistance to cancer formation, particularly in regenerative tissues (Brockes 1998). The local administration of chemical carcinogens during regeneration in these animals results in markedly low tumour incidence (Tsonis 1983; Tsonis and Eguchi 1981), and in particular, tumours of mesenchymal origin never arise (Tsonis and Eguchi 1981; Tsonis and Eguchi 1982; Zilakos et al. 1992). In cases when abnormal regeneration does occur, carcinogen treatment manifests instead in supernumerary regenerates (Eguchi and Watanabe 1973), such as supernumerary appendages or lens formation from the ventral iris, a process never observed during normal lens regeneration. On the basis of these observations, it has been proposed that cells harbouring tumorigenic mutations are tightly constrained within the framework imposed by epimorphic regeneration (Brockes 1998). In addition, cellular senescence could conceivably serve to spatiotemporally restrict enhanced cellular plasticity to within the blastema, and prevent its occurrence outside of regenerative contexts. As such, their elimination by the immune system would represent an important mechanism to constrain tumourigenesis within the highly plastic environment required for regeneration.

3.2.1.4 What About Senescence in Salamander Regeneration?

The discovery of cellular senescence in the blastema represents the first evidence of senescence in *bona fide* regeneration (Yun et al. 2015). Although currently a phenomenological description, the transient induction of cellular senescence in this context shares many features with acute senescence, and as such, raises the possibility that it may be functionally important for regeneration. Senescent cells are able to exert a strong influence on the surrounding microenvironment of the blastema through paracrine signalling via the SASP. Indeed, a strong SASP signature is detected within the blastema coinciding with the peak of senescent cell induction (Yun et al. 2015). Drawing from the known understanding of how cellular senescence is able to impact numerous biological processes, it is possible to formulate a number of hypotheses for how cellular senescence could contribute towards epimorphic regeneration (Fig. 3.3) (Yun 2018). Firstly, senescent cells could directly modulate the behaviour of progenitors, such as mobilising stem cell reserves or enhancing their generation through increased dedifferentiation and proliferation. The fact that senescence induction coincides with the period of generation and expansion of regenerative progenitors is consistent with such a hypothesis, and it will be interesting to assess whether the interplay between cellular senescence and plasticity extends to the context of natural reprogramming. Alternatively, senescent cells could contribute indirectly by establishing

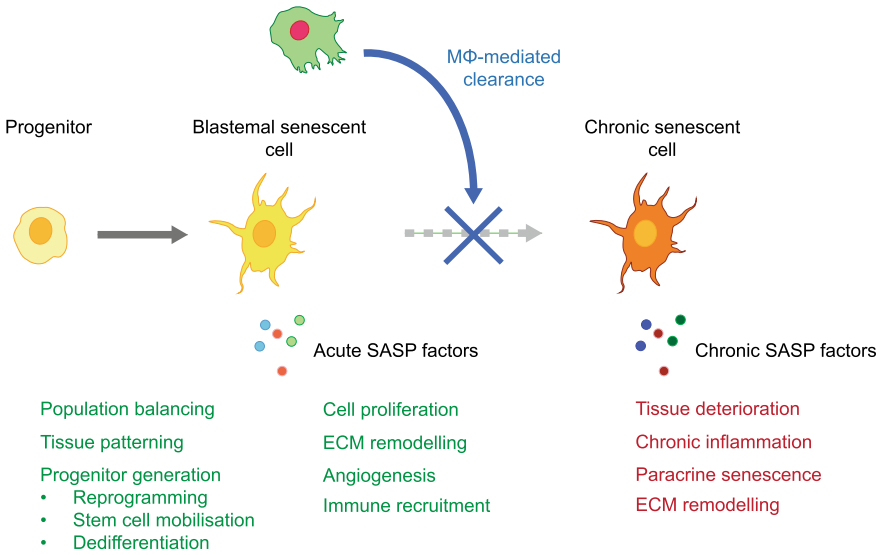

Fig. 3.3 Regenerative effects of senescent cells. Senescent cells are able to impact a variety of biological processes. In the context of epimorphic regeneration, senescence could modulate tissue patterning and morphogenesis, population balancing, contribute to generation of regenerative progenitors, increase in cell proliferation, and angiogenesis. Acute and chronic SASP factors comprise molecules of different nature, or similar factors whose effect may differ due to the strength or kinetics of their expression, or the biological context

a microenvironment conducive for regeneration, such as through matrix remodelling, by stimulating angiogenesis, or through recruiting components of the immune system that in turn exert pro-regenerative functions. Specifically, macrophages are an essential cell type required for salamander regeneration (Godwin et al. 2013) and are found in close proximity to senescent cells in the blastema (Yun et al. 2015). In line with this, it is possible that senescent cells elicit changes in macrophage polarisation and functionality during regeneration, as observed in the context of hepatocellular carcinogenesis (Lujambio et al. 2013). Lastly, senescence could serve as a population balancing mechanism, either in a cell-autonomous manner by restricting the expansion of particular populations, or by acting on neighbouring cells, as observed in the context of developmental senescence in the murine endolymphatic sac (Munoz-Espin et al. 2013). Such balancing would be important for proper patterning, in which cells of multiple lineages must proliferate and differentiate co-ordinately to couple growth and patterning to reconstitute a functional limb.

An expansion in the available experimental tools for both salamander and senescence research (Box 3.1) means it is now possible to directly address these hypotheses experimentally, and thereby delineate the relationship between cellular senescence and regeneration on the molecular and cellular levels. In order to do so, it is necessary to deplete the blastemal senescent population and assess the impact on the outcome of regeneration. The development of pharmacological (Childs et al. 2017) and genetic (Demaria et al. 2014; Baker et al. 2011; Baker et al. 2016) approaches to specifically target senescent cells in vivo enables ablation of the senescent population, making it possible to assess how regeneration occurs in the absence of senescent cells through conventional methods such as histology and live imaging. The same methods can be employed for isolation and detailed characterisation of the blastemal senescent cell population. Such studies will provide important insights into their nature, and address key questions such as their ontogeny, the signalling networks underlying induction of the senescent state, and how they evolve throughout different stages of regeneration. Transcriptional profiling will further provide a platform for functional studies, and identify candidate pro-regenerative SASP factors for functional analysis and senescent-derived signals that mediate immune recruitment and their associated clearance. Moreover, the recent sequencing of the axolotl and newt genomes (Nowoshilow et al. 2018; Elewa et al. 2017) will enable in-depth comparative analysis of senescent-related genes, as well as allowing their genetic manipulation to generate knock-out or knock-in organisms. A better understanding of these processes on the molecular and cellular level is of interest not only to elucidating the mechanism of epimorphic regeneration, but also for therapeutic approaches for regenerative medicine and senescence-based interventions.

3.3 Senescence in Ageing

3.3.1 A Causal Link Between Senescence and Organismal Ageing

An important aspect of the beneficial effects of acute senescence is its transient nature. However, cellular senescence can also have deleterious effects, especially if allowed to persist. It has long been postulated that cellular senescence drives ageing phenotypes, an idea supported by observations of senescent cell accumulation in rodent, primate and human tissues with age (Dimri et al. 1995; Lawless et al. 2010; Wang et al. 2009; Jeyapalan et al. 2007; Herbig et al. 2006). A causal role for senescence in ageing and age-related decline was provided by seminal studies by van Deursen and colleagues. Through the use of a transgene termed INK-ATTAC, which specifically induces apoptosis in p16-expressing cells upon administration of the synthetic compound AP20187, it was shown that selective elimination of senescent cells in BubR1 progeroid mice delayed the onset of several age-related diseases (Baker et al. 2011). Subsequently, the beneficial effects of senescent cell clearance were extended to natural ageing: elimination of p16-positive cells in naturally-aged mice extended median lifespan and attenuated the functional decline of heart, kidney and fat (Baker et al. 2016). Furthermore, treatment delayed cancer progression and resulted in higher spontaneous activity and exploratory behaviour (Baker et al. 2016). Other studies have further reinforced the contribution of cellular senescence to a wide range of age-related diseases, including astherosclerosis, sarcopenia, neurodegeneration and osteoarthritis among others (Jeon et al. 2017; Childs et al. 2016; Sousa-Victor et al. 2014; Bussian et al. 2018).

Mechanistically, cellular senescence is thought to drive ageing through two mechanisms. Firstly, replicative arrest of stem-cell and progenitor pools has been proposed to prevent their participation in tissue regeneration (van Deursen 2014), an idea supported by the observation that progenitor cells of skeletal muscle and fat tissue of BbuR1 progeroid mice are more prone to undergo senescence (Baker et al. 2013) and the demonstration that geriatric muscle stem cells lose their reversible quiescent state during ageing through the induction of senescence (Sousa-Victor et al. 2014; Garcia-Prat et al. 2016). The induction of senescence in muscle satellite cells is coupled with a decline in autophagy during ageing, and consequent loss of proteostasis, mitochondrial dysfunction and the generation of ROS. Specific silencing of p16 in geriatric satellite cells and re-establishment of autophagy enabled cell-cycle re-entry and restored their regenerative functions (Garcia-Prat et al. 2016). Secondly, cellular senescence is able to drive organismal ageing through adverse effects of the SASP. Chronic secretion of cytokines and chemokines can drive sterile inflammation, a hallmark of ageing (Lopez-Otin et al. 2013). SASP-derived proteases are able to cleave membrane-bound receptors, ligands, extracellular matrix proteins or other components in the tissue. In addition, senescence induction can spread across tissues through a mechanism known as paracrine senescence, which is dependent on cytokines such as IL-1ß, TGFß and chemokines (Acosta et al. 2013; Nelson et al.

2012). Together, these processes could disrupt local stem cell niches and overall tissue architecture with age, driving ageing-associated deterioration. Another important consideration is that senescent cells can remain viable in culture for months and continually evolve following the initial cell-cycle arrest, and enter a state termed 'deep' or 'late' senescence (De Cecco et al. 2013). Senescence progression is accompanied by extrusion of chromatin into the cytoplasm to form cytoplasmic chromatin fragments [CCFs] (Ivanov et al. 2013). Lysosome-mediated proteolysis drives histone loss, and is thought to contribute to epigenomic remodelling and SASP diversification in chronic senescent cells. In addition, chronic senescent cells are characterised by a dramatic increase in the transcription of retrotransposable elements [RTEs] (De Cecco et al. 2013). Recent evidence has functionally linked RTE activation with adverse effects of the late SASP (De Cecco et al. 2019). Transcriptional de-repression of L1 RTEs and reduced exonuclease activity results in the accumulation of L1 cytosolic cDNA, which activates the type-I IFN response through the cGAS-STING pathway (De Cecco et al. 2019). Treatment with the nucleoside reverse transcriptase inhibitor lamivudine resulted in dampening of the late SASP response [e.g. expression of CCL2, IL-6 and MMP3] and alleviate several ageing phenotypes in vivo, without impacting cell-cycle arrest or the early SASP response (De Cecco et al. 2019).

3.3.2 Senescence and Ageing in Salamanders

In contrast to mammals, a survey of adult salamander tissues showed a remarkable absence of senescent cells (Yun et al. 2015). Notably, salamanders possess very long lifespans, lack obvious signs of ageing, and are able to sustain indefinite rounds of regeneration throughout their lifetime (Eguchi et al. 2011). Key questions are how salamanders maintain such low systemic levels of senescent cells during ageing, and whether this underlies their lack of age-related deterioration and sustained regenerative capacity. The paucity of senescent cells in salamanders can be conceivably attributed to (Hayflick and Moorhead 1961) active mechanisms to restrict the induction of cellular senescence and/or (van Deursen 2014) efficient clearance mechanisms relative to mammals that persist throughout their lifespan (Fig. 3.3). Clearance refers to the mechanisms by which components of the immune system detect senescent cells and mediate their elimination. Experimental data suggest that both mechanisms contribute towards the low basal levels of senescent cells observed in salamanders (Yun et al. 2015; Ferretti and Brockes 1988). For example, salamander blastemal cells do not undergo crisis or replicative senescence, and can be maintained for more than 200 generations in culture (Ferretti and Brockes 1988), indicating the presence of active mechanisms to circumvent senescence. This property of urodele cells is thought to underlie their capacity to sustain an indefinite number of regeneration cycles.

Studies of cellular senescence in salamanders uncovered a highly robust mechanism for senescent cell surveillance and clearance (Yun et al. 2015). It has been proposed the increase in senescent cell accumulation in mammals with age is driven by an increased decline in immune function (van Deursen 2014). This notion is

supported by studies data from mice with impaired immune-mediated cytotoxicity; perforin-knockout mice display premature accumulation of senescent cells and accelerated ageing phenotypes (Ovadya et al. 2018). In salamanders, implanted senescent cells are efficiently detected and eliminated from adult tissues (Yun et al. 2015). This mechanism is dependent on the innate immune system, as evidenced by the persistence of implanted cells following macrophage depletion with chlodrosome treatment (Yun et al. 2015). Owing to their amenability to live imaging and transplantations, together with the use of transgenic reporter lines or pharmaceutic approaches to label senescent cells and components of the immune system, the salamanders constitute an experimental system to investigate the mechanisms by which the immune system efficiently detects and disposes of senescent cells in vivo (Box 3.1). Molecular characterisation of the senescent population may reveal senescent-derived signals that mediate immune recruitment. A better understanding of the pathways governing this process will lead to the identification of molecular targets for experimental perturbation. By experimentally preventing immune-surveillance of senescent cells, it will be possible to evaluate the contribution of immune-mediated clearance towards maintaining the low levels of senescent cells in the salamander during homeostasis. Furthermore, given the low base-line level of endogenous senescent cells in these organisms, it will be interesting to assess whether experimental accumulation of senescent cells would mirror the age-related deterioration caused by chronic senescence observed in mammals or restrict regenerative capacity.

3.4 Conclusions

Taken together, recent studies addressing the roles of cellular senescence under physiological contexts have underscored the capacity of senescent cells to modulate tissue structure and function. Although not yet established experimentally, we speculate that cellular senescence may be functionally important for regeneration. The dynamics of senescence induction and clearance in the blastema are consistent with that observed during development, wound healing and tissue repair; as such, the blastemal senescent population may influence regeneration through similar mechanisms. It will be of both biological and translational interest to delineate the interactions between the senescent population and other cells in the blastema. Furthermore, the causal link between chronic senescence and age-related decline has galvanized efforts to develop novel therapies against senescent cells. One potential strategy is to enhance the immune response against senescent cells. As salamanders possess a remarkably efficient mechanism for immune-mediated surveillance and disposal of senescent cells, they offer an experimental system for mechanistic investigations into the interaction between such cells and the immune system. We expect that a better understanding of the molecular and cellular mechanisms underlying this process will inform rational interventions for senescence-cell removal. Lastly, research in salamanders will provide deeper insights into the evolutionary origins of cellular senescence, and allow for comparisons across the animal kingdom.

Box 3.1 Molecular toolbox for salamander research

- **Germline transgenesis**. Tools for germline transgenesis in both axolotl (Khattak et al. 2013) and Iberian ribbed newts (Hayashi et al. 2013), based on the I-SceI meganuclease and the Tol2 transposon system, have been essential for obtaining a mechanistic understanding of limb regeneration. Owing to the ease with which salamanders can be bred in the laboratory, it is possible to obtain a substantial number of F_0 individuals harbouring transgenes for experimental investigation.
- **Genome sequence and assembly and CRISPR-mediated gene editing**. The recent sequencing and assembly of the 32-Gb axolotl genome (Nowoshilow et al. 2018) and the 20-Gb *P. waltl* genome (Elewa et al. 2017) provides a rich platform for investigations into the molecular basis of regeneration. Together with CRISPR-mediated gene editing (Elewa et al. 2017; Fei et al. 2018), it is possible to assess candidate genes for functional analysis.
- **Somatic gene delivery methods**. Several different technologies exist for gene delivery in salamander cells and tissues, including electroporation (Yun et al. 2013; Echeverri and Tanaka 2003), and different viral transfection methods (Khattak et al. 2013; Whited et al. 2013; Oliveira et al. 2018).
- **Transplantation**. Salamanders are highly receptive to transplantations without graft rejection. The use of surgical manipulation to transplant cells or tissues combined with several molecular and transgenic technologies have been informative towards understanding key aspects of regeneration, including the identity of cell types in the blastema (Kragl et al. 2009). In addition, such methods can be used to implant exogenously-induced senescent cells into salamanders, as described in Yun et al. 2015.
- **Live imaging**. Many salamander tissues are optically transparent, and highly amenable to live imaging (Currie et al. 2016). Through these approaches, it is possible to follow interactions between senescent cells [endogenously induced or implanted] and the immune system.
- **Chemical screening**. Pre-feeding salamander larvae can be reared in microtitre plates to perform moderate-throughput screening for pharmaceutical compounds (Ponomareva et al. 2015) such as senolytics.

References

Abad M, Mosteiro L, Pantoja C, Canamero M, Rayon T, Ors I et al (2013) Reprogramming in vivo produces teratomas and iPS cells with totipotency features. Nature 502(7471):340–345

Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP et al (2013) A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol 15(8):978–990

Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B et al (2011) Clearance of p16(Ink4a)-positive senescent cells delays ageing-associated disorders. Nature 479(7372):232–236

Baker DJ, Weaver RL, van Deursen JM (2013) p21 both attenuates and drives senescence and aging in BubR1 Progeroid Mice. Cell Rep 3(4):1164–1174

Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J et al (2016) Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature 530(7589):184–189

Ben-Porath I, Weinberg RA (2005) The signals and pathways activating cellular senescence. Int J Biochem Cell Biol 37(5):961–976

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB et al (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279(5349):349–352

Borkham-Kamphorst E, Schaffrath C, Van de Leur E, Haas U, Tihaa L, Meurer SK et al (2014) The anti-fibrotic effects of CCN1/CYR61 in primary portal myofibroblasts are mediated through induction of reactive oxygen species resulting in cellular senescence, apoptosis and attenuated TGF-beta signaling. Bba-Mol Cell Res 1843(5):902–914

Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AHFM, Schlegelberger B et al (2005) Oncogene-induced senescence as an initial barrier in lymphoma development. Nature 436(7051):660–665

Brockes JP (1997) Amphibian limb regeneration: Rebuilding a complex structure. Science 276(5309):81–97

Brockes JP (1998) Regeneration and cancer. Bba-Rev Cancer 1377(1):M1–M11

Brockes JP, Kumar A (2005) Appendage regeneration in adult vertebrates and implications for regenerative medicine. Science 310(5756):1919–1923

Bussian TJ, Aziz A, Meyer CF, Swenson BL, van Deursen JM, Baker DJ (2018) Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. Nature 562(7728):578–582

Campisi J (2013) Aging, cellular senescence, and cancer. Annu Rev Physiol 75:685–705

Chen ZB, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M et al (2005) Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 436(7051):725–730

Childs BG, Baker DJ, Wijshake T, Conover CA, Campisi J, van Deursen JM (2016) Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science 354(6311):472–477

Childs BG, Gluscevic M, Baker DJ, Laberge RM, Marquess D, Dananberg J et al (2017) Senescent cells: an emerging target for diseases of ageing. Nat Rev Drug Discov 16(10):718–735

Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M et al (2005) Tumour biology: senescence in premalignant tumours. Nature 436(7051):642

Coppe JP, Desprez PY, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 5:99–118

Currie JD, Kawaguchi A, Traspas RM, Schuez M, Chara O, Tanaka EM (2016) Live Imaging of Axolotl digit Regeneration reveals Spatiotemporal Choreography of Diverse Connective Tissue Progenitor Pools. Dev Cell 39(4):411–423

Czarkwiani A, Yun MH (2018) Out with the old, in with the new: senescence in development. Curr Opin Cell Biol 55:74–80

Davaapil H, Brockes JP, Yun MH (2017) Conserved and novel functions of programmed cellular senescence during vertebrate development. Development 144(1):106–114

De Cecco M, Criscione SW, Peckham EJ, Hillenmeyer S, Hamm EA, Manivannan J et al (2013) Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. Aging Cell 12(2):247–256

De Cecco M, Ito T, Petrashen AP, Elias AE, Skvir NJ, Criscione SW et al (2019) L1 drives IFN in senescent cells and promotes age-associated inflammation (vol 566, pg 73, 2019). Nature 572(7767):73–78

Del R-TK, Tsonis PA (2003) Eye regeneration at the molecular age. Dev Dyn 226(2):211–224

Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR et al (2014) An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell 31(6):722–733

Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C et al (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 444(7119):638–642

Dimri GP, Lee XH, Basile G, Acosta M, Scott C, Roskelley C et al (1995) A biomarker that identifies senescent human-cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 92(20):9363–9367

Dorr JR, Yu Y, Milanovic M, Beuster G, Zasada C, Dabritz JH et al (2013) Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Nature 501(7467):421–425

Echeverri K, Tanaka EM (2003) Electroporation as a tool to study in vivo spinal cord regeneration. Dev Dynam 226(2):418–425

Echeverri K, Clarke JDW, Tanaka EM (2001) In vivo imaging indicates muscle fiber dedifferentiation is a major contributor to the regenerating tail blastema. Dev Biol 236(1):151–164

Eguchi G, Shingai R (1971) Cellular analysis on localization of lens forming potency in newt iris epithelium. Dev Growth Differ 13(4):337–349

Eguchi G, Watanabe K (1973) Elicitation of Lens Formation from Ventral Iris Epithelium of Newt by a Carcinogen, N-Methyl-N'-Nitro-N-Nitrosoguanidine. J Embryol Exp Morph 30(1):63–71

Eguchi G, Abe SI, Watanabe K (1974) Differentiation of lens-like structures from newt iris epithelial-cells In vitro. Proc Natl Acad Sci USA 71(12):5052–5056

Eguchi G, Eguchi Y, Nakamura K, Yadav MC, Millan JL, Tsonis PA (2011) Regenerative capacity in newts is not altered by repeated regeneration and ageing. Nat Commun 2:384

Elewa A, Wang H, Talavera-Lopez C, Joven A, Brito G, Kumar A et al (2017) Reading and editing the Pleurodeles waltl genome reveals novel features of tetrapod regeneration. Nat Commun 8(1):2286

Fei JF, Lou WPK, Knapp D, Murawala P, Gerber T, Taniguchi Y et al (2018) Application and optimization of CRISPR-Cas9-mediated genome engineering in axolotl (Ambystoma mexicanum). Nat Protoc 13(12):2908–2943

Ferretti P, Brockes JP (1988) Culture of Newt Cells from different tissues and their expression of a regeneration-associated antigen. J Exp Zool 247(1):77–91

Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortet L, Rodriguez-Ubreva J, Rebollo E et al (2016) Autophagy maintains stemness by preventing senescence. Nature 529(7584):37–42

Godwin JW, Pinto AR, Rosenthal NA (2013) Macrophages are required for adult salamander limb regeneration. Proc Natl Acad Sci USA 110(23):9415–9420

Gurtner GC, Werner S, Barrandon Y, Longaker MT (2008) Wound repair and regeneration. Nature 453(7193):314–321

Hayashi T, Yokotani N, Tane S, Matsumoto A, Myouga A, Okamoto M et al (2013) Molecular genetic system for regenerative studies using newts. Dev Growth Differ 55(2):229–236

Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25(3):585–621

Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006) Cellular senescence in aging primates. Science 311(5765):1257

Ivanov A, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS et al (2013) Lysosome-mediated processing of chromatin in senescence. J Cell Biol 202(1):129–143

Jeon OH, Kim C, Laberge RM, Demaria M, Rathod S, Vasserot AP et al (2017) Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat Med 23(6):775–781

Jeyapalan JC, Ferreira M, Sedivy JA, Herbig U (2007) Accumulation of senescent cells in mitotic tissue of aging primates. Mech Ageing Dev 128(1):36–44

Jun JI, Lau LF (2010) The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nat Cell Biol 12(7):676–685

Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G et al (2013) A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature 498(7452):109–112

Khattak S, Schuez M, Richter T, Knapp D, Haigo SL, Sandoval-Guzman T et al (2013a) Germline transgenic methods for tracking cells and testing gene function during regeneration in the axolotl. Stem Cell Rep 1(1):90–103

Khattak S, Sandoval-Guzman T, Stanke N, Protze S, Tanaka EM, Lindemann D (2013b) Foamy virus for efficient gene transfer in regeneration studies. BMC Dev Biol 13:17

Kim HM, Wangemann P (2011) Epithelial cell stretching and luminal acidification lead to a retarded development of stria Vascularis and deafness in mice lacking Pendrin. PLoS ONE 6(3):e17949

Kim KH, Chen CC, Monzon RI, Lau LF (2013) Matricellular Protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic Myofibroblasts. Mol Cell Biol 33(10):2078–2090

Kim KH, Won JH, Cheng N, Lau LF (2018) The matricellular protein CCN1 in tissue injury repair. J Cell Commun Signal 12(1):273–279

Kragl M, Knapp D, Nacu E, Khattak S, Maden M, Epperlein HH et al (2009) Cells keep a memory of their tissue origin during axolotl limb regeneration. Nature 460(7251):60–65

Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C et al (2008) Senescence of activated stellate cells limits liver fibrosis. Cell 134(4):657–667

Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci USA 98(21):12072–12077

Kumar A, Velloso CP, Imokawa Y, Brockes JP (2000) Plasticity of retrovirus-labelled myotubes in the newt limb regeneration blastema. Dev Biol 218(2):125–136

Kumar A, Velloso CP, Imokawa Y, Brockes JP (2004) The regenerative plasticity of isolated urodele myofibers and its dependence on Msx1. PLoS Biol 2(8):1168–1176

Laberge RM, Awad P, Campisi J, Desprez PY (2012) Epithelial-mesenchymal transition induced by senescent fibroblasts. Cancer Microenviron 5(1):39–44

Lawless C, Wang CF, Jurk D, Merz A, von Zglinicki T, Passos JF (2010) Quantitative assessment of markers for cell senescence. Exp Gerontol 45(10):772–778

Lo DC, Allen F, Brockes JP (1993) Reversal of muscle differentiation during urodele limb regeneration. Proc Natl Acad Sci USA 90(15):7230–7234

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153(6):1194–1217

Lujambio A, Akkari L, Simon J, Grace D, Tschaharganeh DF, Bolden JE et al (2013) Non-cell-autonomous tumor suppression by p53. Cell 153(2):449–460

Meyer K, Hodwin B, Ramanujam D, Engelhardt S, Sarikas A (2016) Essential role for premature senescence of Myofibroblasts in myocardial fibrosis. J Am Coll Cardiol 67(17):2018–2028

Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM et al (2005) BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 436(7051):720–724

Moiseeva O, Mallette FA, Mukhopadhyay UK, Moores A, Ferbeyre G (2006) DNA damage signaling and p53-dependent senescence after prolonged beta-interferon stimulation. Mol Biol Cell 17(4):1583–1592

Mosteiro L, Pantoja C, Alcazar N, Marion RM, Chondronasiou D, Rovira M, et al (2016) Tissue damage and senescence provide critical signals for cellular reprogramming in vivo. Science 354(6315):aaf4445

Munoz-Espin D, Serrano M (2014) Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Bio 15(7):482–496

Munoz-Espin D, Canamero M, Maraver A, Gomez-Lopez G, Contreras J, Murillo-Cuesta S et al (2013) Programmed cell senescence during mammalian embryonic development. Cell 155(5):1104–1118

Nacu E, Tanaka EM (2011) Limb Regeneration: a new development? Annu Rev Cell Dev Bi 27:409–440

Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA et al (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113(6):703–716

Narita M, Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA et al (2006) A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 126(3):503–514

Nelson G, Wordsworth J, Wang C, Jurk D, Lawless C, Martin-Ruiz C et al (2012) A senescent cell bystander effect: senescence-induced senescence. Aging Cell 11(2):345–349

Nowoshilow S, Schloissnig S, Fei JF, Dahl A, Pang AWC, Pippel M et al (2018) The axolotl genome and the evolution of key tissue formation regulators. Nature 554(7690):50–55

Oliveira CR, Lemaitre R, Murawala P, Tazaki A, Drechsel DN, Tanaka EM (2018) Pseudo typed baculovirus is an effective gene expression tool for studying. Dev Biol 433(2):262–275

Ovadya Y, Landsberger T, Leins H, Vadai E, Gal H, Biran A, et al (2018) Impaired immune surveillance accelerates accumulation of senescent cells and aging. Nat Commun 9(1):5435

Ponomareva LV, Athippozhy A, Thorson JS, Voss SR (2015) Using Ambystoma mexicanum (Mexican axolotl) embryos, chemical genetics, and microarray analysis to identify signaling pathways associated with tissue regeneration. Comp Biochem Phys C 178:128–135

Rajagopalan S, Long EO (2012) Cellular senescence induced by CD158d reprograms natural killer cells to promote vascular remodeling. Proc Natl Acad Sci USA 109(50):20596–20601

Reyer RW, Woolfitt RA, Withersty LT (1973) Stimulation of lens regeneration from newt dorsal iris when implanted into blastema of regenerating limb. Dev Biol 32(2):258–281

Ritschka B, Storer M, Mas A, Heinzmann F, Ortells MC, Morton JP et al (2017) The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. Genes Dev 31(2):172–183

Sedelnikova OA, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, Barrett JC (2004) Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nat Cell Biol 6(2):168–170

Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88(5):593–602

Shah PP, Donahue G, Otte GL, Capell BC, Nelson DM, Cao K et al (2013) Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape. Genes Dev 27(16):1787–1799

Sousa-Victor P, Gutarra S, Garcia-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V et al (2014) Geriatric muscle stem cells switch reversible quiescence into senescence. Nature 506(7488):316–421

Stein GH, Beeson M, Gordon L (1990) Failure to phosphorylate the retinoblastoma gene product in senescent human fibroblasts. Science 249(4969):666–669

Storer M, Mas A, Robert-Moreno A, Pecoraro M, Ortells MC, Di Giacomo V et al (2013) Senescence is a developmental mechanism that contributes to embryonic growth and patterning. Cell 155(5):1119–1130

Tanaka EM (2016) The molecular and cellular choreography of appendage regeneration. Cell 165(7):1598–1608

Tsonis PA (1983) Effects of carcinogens on regenerating and non-regenerating limbs in amphibia. Anticancer Res 3(3):195–202

Tsonis PA, Eguchi G (1981) Carcinogens on regeneration—effects of N-Methyl-N'-Nitro-N-Nitrosoguanidine and 4-Nitroquinoline-1-Oxide on limb regeneration in adult newts. Differentiation 20(1):52–60

Tsonis PA, Eguchi G (1982) Abnormal limb regeneration without tumor production in adult newts directed by Carcinogens, 20-Methylcholanthrene and Benzo (Alpha) Pyrene. Dev Growth Differ 24(2):183–190

van Deursen JM (2014) The role of senescent cells in ageing. Nature 509(7501):439–446

Villiard E, Denis JF, Hashemi FS, Igelmann S, Ferbeyre G, Roy S (2017) Senescence gives insights into the morphogenetic evolution of anamniotes. Biol Open 6(6):891–896

von Zglinicki T (2002) Oxidative stress shortens telomeres. Trends Biochem Sci 27(7):339–344

Wang CF, Jurk D, Maddick M, Nelson G, Martin-Ruiz C, von Zglinicki T (2009) DNA damage response and cellular senescence in tissues of aging mice. Aging Cell 8(3):311–323

Wang JW, Geiger H, Rudolph KL (2011) Immunoaging induced by hematopoietic stem cell aging. Curr Opin Immunol 23(4):532–536

Whited JL, Tsai SL, Beier KT, White JN, Piekarski N, Hanken J et al (2013) Pseudotyped retroviruses for infecting axolotl in vivo and in vitro. Development 140(5):1137–1146

Yun MH (2015) Changes in regenerative capacity through lifespan. Int J Mol Sci 16(10):25392–25432

Yun MH (2018) Cellular senescence in tissue repair: every cloud has a silver lining. Int J Dev Biol 62(6–8):591–604

Yun MH, Gates PB, Brockes JP (2013) Regulation of p53 is critical for vertebrate limb regeneration. Proc Natl Acad Sci USA 110(43):17392–17397

Yun MH, Davaapil H, Brockes JP (2015) Recurrent turnover of senescent cells during regeneration of a complex structure. Elife 4:e05505

Zhang R, Chen W, Adams PD (2007) Molecular dissection of formation of senescence-associated heterochromatin foci. Mol Cell Biol 27(6):2343–2358

Zilakos NP, Tsonis PA, Delriotsonis K, Parchment RE (1992) Newt squamous carcinoma proves phylogenetic conservation of tumors as caricatures of tissue renewal. Cancer Res 52(18):4858–4865