

# **Short-term Gain, Long-term Pain: The Senescence Life Cycle and Cancer**

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## **Summary**

Originally thought of as a stress response end-point, the view of cellular senescence has since evolved into one encompassing a wide range of physiological and pathological functions, including both pro- and anti-tumorigenic features. It has also become evident that senescence is a highly dynamic and heterogeneous process. Efforts to reconcile the beneficial and detrimental features of senescence suggest that physiological functions require the transient presence of senescent cells in the tissue microenvironment. Here, we propose the concept of a physiological 'senescence life cycle', which has pathological consequences if not executed to its entirety.

## Introduction

The concept of senescence has been evolving over recent decades, having originally been described as a stress response that leads to a persistent proliferative arrest even in the presence of growth factors. Since senescence can limit the propagation of damaged cells (which are at risk of malignant transformation), removing them from the proliferative pool (Campisi 2001; Lowe et al. 2004), it has therefore been considered to be an endogenous tumor suppressor mechanism or even a therapeutic goal, alternative to apoptosis. However, this is not an all-encompassing view of senescence. Known as the senescence-associated secretory phenotype (SASP), senescent cells typically secrete diverse factors, which affect the tumor microenvironment. This is also important for stromal cell senescence, which can be either anti- or pro-tumorigenic depending on the context (reviewed in (Pérez-Mancera et al. 2014)). It is perhaps safe to say that senescence is an autonomous tumor suppressor but a non-autonomous tumor 'modulator' (Hoare et al. 2018).

Senescence has also been implicated in aging as one of the hallmarks of aging (López-Otín et al. 2013). It has been proposed that the age-dependent accumulation of senescent cells within tissue stem or progenitor cell compartments results in the decline of their regenerative capacity in multiple tissues (reviewed in (Sharpless and DePinho 2007)). These studies have provided the first functional relevance for senescence in aging within body organs, but more recent evidence that enforced killing of senescent cells in mice extends their life span has firmly established the causal role of senescence in aging (Baker et al. 2011; 2016).

In parallel, recent evidence has described more physiological roles for senescent cells that extend beyond the cancer and aging contexts, with functions in shaping embryonic development (Storer et al. 2013; Muñoz-Espín et al. 2013; Chuprin et al. 2013), contributing to the maturation of certain cell types (Besancenot et al. 2010; Helman et al. 2016) and maintaining tissue integrity in adult organisms by facilitating wound healing (Berg et al. 2003; Krizhanovsky et al. 2008; Jun and Lau 2010a; Demaria et al. 2014). Thus, despite once being considered a state of ‘functional death’, senescent cells do have diverse functionalities *in vivo*.

These functions of senescent cells in tumor suppression or tissue homeostasis are often reinforced by or dependent on their transient presence, respectively, within the microenvironment, where senescent cells are eventually removed by immune cells (Ito et al. 2017). At least in some contexts, senescence can be seen as a progressive process, initiated by cellular activation, developing senescence effectors, modulation of the tissue microenvironment and, finally, the recruitment of immune cells to mediate resolution (Krizhanovsky et al. 2008). Senescence-associated disorders can perhaps be viewed as a consequence of a failure in the execution of the ‘senescence life cycle’ (Fig. 1). Misregulation of senescence within tissues can promote pathological conditions including cancer and other age-related disorders (Burton and Krizhanovsky 2014; Muñoz-Espín and Serrano 2014; He and Sharpless 2017). In this review, we provide an overview of the relevance of senescence in tumor development, discussing both the autonomous and the non-autonomous aspects of senescence. We recently reviewed the relationship between senescence and cancer hallmarks elsewhere (Hoare et al. 2018). Here, we focus on the various aspects of the senescence

response and discuss the implication of senescence in tumor development and treatment with particular emphasis on early stage tumors.

### **Senescence as heterogeneous phenotype**

The first observation of cellular senescence *in vitro* was described in replicative exhaustion, now known to be the result of telomere shortening (Hayflick 1965; Shay and Wright 2000). Since then, various stress-associated inducers of 'premature' senescence have been identified, including DNA damage, oxidative stress, aberrant oncogene activation, and tumor suppressor gene loss (Kuilman et al. 2010; Campisi 2013). These cellular stressors evoke persistent DNA damage signalling and activation of the p53/p21 and p16/pRB tumor suppressor pathways, which mediate entry into, and maintenance of, senescence (d'Adda di Fagagna et al. 2003; Mallette et al. 2007; Rodier et al. 2009; Campisi and d'Adda di Fagagna 2007). Furthermore, a number of additional effector programs are involved in the senescence process, and the concept of senescence as a collective phenotype of multiple effectors, instead of a single entity, has been proposed. As such, due to the lack of a single biomarker that is both specific and unique to senescence, it is typically characterized using a combination of associated cellular changes, including chromatin re-organization, the gene expression profile, and secretome and metabolic pathways (Salama et al. 2014; Kuilman et al. 2010). In light of this, using an analogy from a clinical term, entry into senescence can perhaps be viewed as a 'syndrome': the incremental acquisition of associated phenotypes instead of a binary event where a defined set of changes manifest at the same time. This is consistent with the previously proposed concept of the differing depths of the senescence phenotype, which is modified by important

cellular changes that follow an initial cell cycle arrest (Baker and Sedivy 2013; Chen and Ozanne 2006; Passos et al. 2010; Ivanov et al. 2013). This may further explain the well-described phenotypic heterogeneity of senescent cells, which manifests from the level of gene expression, where the phenotype depends on cell-type, context, and nature of the upstream stressor (Passos et al. 2007; van Deursen 2014; Marthandan et al. 2016; Hernandez-Segura et al. 2017). Senescent cell populations have also been described to possess a high degree of cell-to-cell variability that exceeds that of quiescent cells (Wiley et al. 2017), reinforcing the heterogeneity of senescence even at the single cell level.

### **Senescence in tumors**

As the induction of senescence is essentially the converse of the canonical unrestricted proliferation that defines cancer cells, much of the original research into the biology of senescence focused on its role in endogenous tumor suppression, which limits immortalization (telomere shortening induced senescence, see next section) and excessive mitotic signaling (oncogene-induced senescence, OIS).

#### *Telomere shortening*

The tumor suppressive role of senescence was originally conceived in the context of telomere dysfunction (Shay and Wright 2005). Replicative senescence, for instance, can be considered to be a counterpart of immortalization in culture. Indeed, replicative senescence can be, at least partially, rescued by ectopic expression of telomerase in culture (Bodnar et al. 1998; Vaziri and Benchimol 1998) and most (>90%) human cancers aberrantly upregulate telomerase (Kim et al. 1994; Shay and Bacchetti 1997; Jafri et al. 2016). The tumor suppressive role of telomere shortening is recapitulated

in telomerase deficiency in various tumor-prone mouse models (González-Suárez et al. 2000; Blasco 2005). However, telomerase deficiency alone in mice can lead to an increase in spontaneous cancer incidence mainly in rapidly proliferating cell types, exemplified by the unusually high incidence of teratocarcinomas, probably due to increased genome instability, a hallmark of cancer (Rudolph et al. 1999). Importantly, these exceptions, where short telomeres counter-intuitively promote the incidence of cancer, tend to be associated with a p53 defect (Chin et al. 1999; Artandi et al. 2000).

This is consistent with the view that telomere dysfunction triggers a DNA damage response, which activates the p53 pathway (d'Adda di Fagagna et al. 2003; Deng et al. 2008). The downstream effects of this pathway can be apoptosis and/or senescence. However, which component(s) are responsible for the tumor suppressive activity of short telomeres *in vivo*? Using a mouse model of Burkitt's lymphoma with telomerase deficiency (Adams et al. 1985; Blasco et al. 1997), Feldser and Greider report that apoptosis is dispensable for short telomere-mediated tumor suppression. Provided that p53 is intact, mice remain resistant to tumor formation even following the blockade of apoptosis. Instead, micro-lymphomas, which stain positive for markers of senescence are observed, demonstrating the importance of senescence as an effector of tumor suppression in this context (Feldser and Greider 2007).

These data suggest that the intrinsic limitation of proliferative capacity can contribute to a reduction in both tissue regenerative capacity and cancer initiation (Liu et al. 2011; Chang 2005). This appears to support the idea of a trade-off between aging and cancer (Kirkwood and Austad 2000; Tyner et al. 2002), although this view only

considers the autonomous aspect of senescence (see 'Non-autonomous effectors' below) and telomere shortening is just one way to trigger senescence.

Telomeres are notorious for DNA repair: it has been suggested that DNA damage in telomeres is effectively unrepairable, causing a persistent DNA damage response (DDR) and the formation of telomere-associated foci (TAF) in ageing and stress-induced senescence (Fumagalli et al. 2012; Hewitt et al. 2012; Takai et al. 2003). This can occur independently of telomere length, as markers of DDR can accumulate at telomeres that are not yet critically shortened (Fumagalli et al. 2012). Although the chromosome body can also harbor persistent DNA damage after genotoxic stress (called DNA segments with chromatin alterations reinforcing senescence, DNA-SCARS) (Rodier et al. 2010), live cell imaging experiments have previously suggested that the majority of these persistent foci are associated with telomeres (Hewitt et al. 2012). These residual DNA damage foci appear to contribute to the maintenance of cell cycle arrest (Passos et al. 2010; Rodier et al. 2010) suggesting that telomere dysfunction-mediated senescence can also occur in contexts other than replicative stress.

Importantly, DNA damage can induce senescence even in cancer cells in some contexts: called therapy-induced senescence (TIS) (Schmitt et al. 2002; Nardella et al. 2011; Acosta and Gil 2012), and TAF formation after irradiation is not affected by telomerase activity (Fumagalli et al. 2012; Hewitt et al. 2012), suggesting that cancer cells could possibly be subjected to this mechanism. However, the anti-tumor effect of TIS is less evident and highly context and model dependent (Dörr et al. 2013; Tato-Costa et al. 2016; Demaria et al. 2017; Yang et al. 2017b). This is perhaps in part due



to the diversity of the SASP components and their pleiotropic activities, as well as the heterogeneity and stochasticity of cancers and their response to therapies within the tissue microenvironment. This point is further discussed below (see 'Non-autonomous effectors').

### *Senescence in preneoplasia*

While replicative senescence limits cellular 'immortalization', an early step in tumorigenesis, an alternative autonomous tumor suppressive function of senescence can be seen in OIS, where some 'mitogenic' oncogenes (or loss of tumor suppressors, as exemplified by PTEN loss-induced cellular senescence, PICS) can paradoxically induce persistent proliferative arrest (Braig and Schmitt 2006; Courtois-Cox et al. 2008; Pérez-Mancera et al. 2014). OIS was first described in human diploid fibroblasts in culture, where a phenotype similar to replicative senescence was observed following ectopic expression of constitutively active HRas (Serrano et al. 1997) or its downstream effectors MEK (Lin et al. 1998) or RAF1 (Zhu et al. 1998). This senescent phenotype is accompanied by accumulation of the tumor suppressor proteins p53 and p16 and a concomitant ablation of these tumor suppressors leads instead to transformation, supporting the notion of OIS as an intrinsic tumor suppressor mechanism (Lin et al. 1998; Serrano et al. 1997). In mouse models, the tumor suppressor p19Arf has been shown to mediate the activation of p53 in response to oncogenic Ras (Palmero et al. 1998). Following the description of OIS *in vitro*, senescent cells harboring oncogenic mutations were observed to accumulate in premalignant lesions of various tissue types both in humans and mice (Collado et al. 2005; Michaloglou et al. 2005; Chen et al. 2005; Braig et al. 2005; Lazzerini Denchi et al. 2005; Collado and Serrano 2010; Narita and Lowe 2005).

In a cell-autonomous manner, the nature of the activating oncogenic signal influences the balance between entry into senescence and tumorigenesis, where oncogene dose and the presence of other cooperating mutations have been suggested to influence cellular outcome (Sarkisian et al. 2007; Quintanilla et al. 1986; Tuveson et al. 2004). The most widely used OIS model remains the classic culture model: overexpression of oncogenic HRAS-G12V-induced senescence in human diploid fibroblasts (Serrano et al. 1997). This is highly robust and, particularly with the introduction of a 4OHT-inducible system (Young et al. 2009), useful for procedures which require a large number of cells, as are often required for genomic and proteomic approaches. However, one inherent caveat of this model is the level of Ras, which is supra-physiological. The natural Ras-driven tumorigenesis is typically initiated with a single allelic oncogenic mutation (Hobbs et al. 2016).

Evidence for the notion that oncogenic dose influences cellular outcome was provided by earlier studies showing that mouse embryonic fibroblasts (MEFs) expressing endogenous level of the single allelic *Kras-G12V* or *Kras-G12D* fail to undergo senescence, although they were not fully transformed (Guerra et al. 2003; Tuveson et al. 2004). A similar insensitivity to senescence-induction by a moderate level of ectopic oncogenic Ras or its downstream effector, Raf was also shown in human- or immortalized mouse-fibroblasts (Deng et al. 2004; Sewing et al. 1997). At the same time, mouse models developed to recapitulate the role of oncogenic Ras mutations in tumorigenesis have shown that, while endogenous *Kras-G12V* expression leads to premalignant lesions in the pancreas (Hingorani et al. 2003) and preneoplastic hyperplasia in the lung and intestine, additional cellular alterations are typically

required for progression to full malignancy (Tuveson et al. 2004). Furthermore, only a subset of cells are transformed by oncogenic KRas, even then in a highly context-dependent manner (Jackson et al. 2001; Guerra et al. 2003; DuPage et al. 2009; Lee and Bae 2016).

These observations suggest that a low dose of oncogenic Ras is not sufficient to trigger senescence programs nor malignant transformation. This was further supported by a mouse breast cancer model, where the level of a doxycycline-inducible ectopic *Hras-G12V* can be titrated (Sarkisian et al. 2007). Consistent with the earlier studies, this study also showed that high- but not low-level Ras induces senescence in mammary glands *in vivo*. In addition, while low-level Ras (comparable to the level expressed from the endogenous *Kras-G12D* allele in the mouse pancreas model described above) is not immediately sufficient for cancer development, the mice eventually develop tumors. Interestingly, these tumors (derived from low-level Ras) are accompanied by the spontaneous upregulation of oncogenic Ras, to a level similar to that of high-level Ras, which induces senescence. Furthermore, they observed senescent mosaicism within those low-Ras initiated tumors with spontaneous upregulation of oncogenic Ras. Mechanisms for the spontaneous upregulation of oncogenic Ras in this study were not clear, but a similar upregulation of oncogenic Ras during cancer development has been reported in different tumor models (Quintanilla et al. 1986; Finney and Bishop 1993; Aguirre et al. 2003; Junttila et al. 2010).

It is possible that, even when it is initiated by a single copy mutation, Ras activity needs to be increased for full malignant transformation but that this is counteracted by

senescence programs. Perhaps the OIS culture system models a tumor suppressive event at this critical step of Ras-driven tumorigenesis (Fig. 2). Of note, the Ras pathway is regulated by diverse effectors, thus its oncogenic activity can be upregulated through multiple routes (Downward 2003; Calvisi et al. 2006; Courtois-Cox et al. 2006; Shaw et al. 2007; Vandal et al. 2014). It would be important to determine the correlation between the level of oncogenic activity and the senescence phenotype during the preneoplastic stage in those genetically engineered OIS models.

### **Autonomous senescence effectors**

As a collective phenotype made up of numerous cellular effector programs, we discuss autonomous and non-autonomous effectors separately, and here we focus on chromatin and genomic alterations as representative of the autonomous effectors potentially contributing to the static nature of senescence arrest.

#### *Epigenetics*

It has been proposed that senescence, unlike quiescence (a state of physiological and readily reversible cell cycle arrest), employs distinct alterations in the chromatin landscape (Parry and Narita 2016). These epigenetic and chromatin alterations occur at various levels: including DNA methylation, histone marks and variants, chromatin accessibility and non-coding RNAs (Buschbeck and Hake 2017; Pal and Tyler 2016; Parry and Narita 2016; Nacarelli et al. 2017). Among these, DNA methylation (5-Methylcytosine at CpG) is, on the one hand, known as a marker of constitutive heterochromatin, particularly at regions with repetitive sequences. On the other hand, although CpG islands, which are abundant regulatory elements in mammalian promoters, are usually hypomethylated, the CpG islands of some genes can be

hypermethylated in abnormal conditions: e.g. hypermethylation of CpG islands at the promoters of tumor suppressors leads to their silencing and promotes tumorigenesis (Deaton and Bird 2011).

It has been long known that DNA methylation globally declines during senescence, forming the basis for the well-known 'heterochromatin loss model': the idea that a gradual breakdown of heterochromatin leads to the de-silencing of otherwise repressed genes (or non-coding RNAs), contributing to senescence and aging (Villeponteau 1997). However, more recent studies using next generation sequencing technology have revealed that the alterations in DNA methylation during senescence are not unidirectional. Using a model of replicative senescence in human fibroblasts, Cruickshanks et al. demonstrated that DNA methylation is globally reduced at heterochromatic regions, but focally increased at a subset of CpG islands. Interestingly, the pattern of hypermethylated CpG islands in replicative senescence is reminiscent of that of cancers, leading to the hypothesis that senescence and cancer share features of epigenetic change. This suggests that cells which have escaped from senescence would be more susceptible to subsequent transformation (Cruickshanks et al. 2013).

This idea, that senescence-associated epigenetic alterations might contribute to a tumor suppressive senescence phenotype but at the same time encompass oncogenic properties, appears to explain well the dual effect of senescence in cancer, i.e. senescence as a tumor suppressor and yet also as a risk factor for cancer. This might also provide a partial explanation for the recent study suggesting that senescence escapers exhibit a stem-like signature with high tumor initiating potential

(Milanovic et al. 2018). However, a recent study provides an alternative view. By directly comparing the DNA methylation profile between replicative senescent and transformed cells derived from the same cell line, Xie et al. identified a global reduction with focal increases in DNA methylation during senescence, consistent with the findings of the previous study. However, looking at individual genomic regions conversely suggested that methylation patterns evolved separately during tumorigenesis and progression to senescence: the former being stochastic and the latter being highly reproducible and thus suggesting that they are somewhat programmed. Based on this data, the authors propose that transformation-associated changes in DNA methylation status do not stem from senescence escape but rather reflect alterations which can be associated independently with both aging and increased cancer risk (Xie et al. 2018). In contrast to replicative senescence, OIS-associated DNA methylation alterations appear to be minimal, underscoring the diversity of the senescence phenotype (Xie et al. 2018; Sakaki et al. 2017). Whichever is the case, the decline in DNA methylation of heterochromatic regions with repetitive sequences (e.g. transposons, satellite DNAs) in senescence or cancer potentially causes genome instability through a reactivation of transposons for instance. Indeed, expression of retrotransposable elements and satellite sequences have been shown to increase during replicative senescence (De Cecco et al. 2013b).

In an interesting contrast to DNA methylation, the profile of DNA accessibility (a marker for open chromatin, which can be an indicator of developmental maturity (Stergachis et al. 2013)) is also altered during replicative senescence: DNA accessibility exhibits a global increase with focal declines (De Cecco et al. 2013a). This appears to show a mirror image of the DNA methylation pattern, but whether or not DNA methylation and

chromatin accessibility are physically or functionally related has not been tested. In OIS too, a general increase in chromatin accessibility has been reported, and different oncogenes induce different profiles of accessible regions upon senescence entry (Parry et al. 2018)

### *Chromatin dynamics*

In addition to the alterations to the linear structure of the genome, widespread changes in higher-order chromatin structure have been observed during senescence. For example, senescence-associated heterochromatic foci (SAHF) are readily visualized by microscopy as DAPI-dense foci in the nucleus (Narita et al. 2003; Zhang et al. 2005). A number of structural and functional factors that are required for SAHF formation have been identified: e.g. HMGA chromatin architectural proteins and histone co-chaperones HIRA/ASF1a have been shown to be necessary for SAHF formation, whereas loss of linker histone H1 is correlated with SAHFs (Zhang et al. 2005; Narita et al. 2006; Funayama et al. 2006). It was initially proposed that SAHFs are involved in the stable silencing of a subset of genes, including cell cycle genes (Narita et al. 2003; Sadaie et al. 2013; Zhang et al. 2007), but it is also possible that inter-SAHF euchromatic regions actively take part in stable gene activation. It is important to note that SAHF formation is model dependent, which may represent the heterogeneity of senescence. Interestingly, higher levels of p16 and HMGA, both of which are critical for SAHF formation, are correlated with the irreversibility of the senescence arrest (Beauséjour et al. 2003; Narita et al. 2006), reinforcing the existence of a spectrum of senescence, an idea associated with the 'senescence syndrome' mentioned above.

In contrast to SAHF, senescence-associated distension of satellites (SADS) represent a distension of  $\alpha$ -satellite and satellite II sequences, which are heterochromatic and normally compacted at the (peri)centromere, during senescence. SADS appear to be more universal than SAHFs (Swanson et al. 2013). Interestingly, both of these newly formed heterochromatin (SAHFs) and unfolding of constitutive heterochromatin (SADS) appear not to require alterations in repressive marks, H3K9me3 and H3K27me3 (Chandra et al. 2012; Swanson et al. 2013). Instead, these chromatin structural changes are mediated by a differential association with the nuclear membrane. In proliferating cells, heterochromatin typically localizes preferentially to the nuclear periphery and around the nucleolus, tethered by lamina-associated and nucleolus-associated chromatin domains (LADs and NADs respectively) (Padeken and Heun 2014). While NADs have been suggested to be conserved at least in replicative senescence (Dillinger et al. 2017), the level of Lamin B1 declines during senescence (Shimi et al. 2011; Freund et al. 2012; Sadaie et al. 2013; Shah et al. 2013; Criscione et al. 2016). Although the loss of Lamin B1 is not sufficient for senescence or SAHF induction, it liberates H3K9me3-rich heterochromatin from the nuclear periphery and facilitates SAHF formation (Sadaie et al. 2013). These observations have led to the model whereby SAHFs are formed through the spatial re-positioning of pre-existing repressive histone marks (Chandra et al. 2012; 2015). Although it is not clear how Lamin B1 loss promotes SADS formation, it has been shown that SADS often extend along or toward the nuclear periphery and the researchers speculated that alterations in Lamin B1 and other structural proteins might contribute to this process (Swanson et al. 2013).



Another consequence of Lamin B1 downregulation during senescence is compromised nuclear envelope integrity and the subsequent leakage of chromatin fragments from the nucleus into the cytoplasm as cytoplasmic chromatin fragments (CCFs). Although it was reported that Lamin B1 downregulation during senescence is mediated by RB-dependent silencing (Shimi et al. 2011), Lamin B1 is also a substrate of autophagic degradation (see below) (Dou et al. 2015; Lenain et al. 2015). Indeed, CCFs, together with Lamin B1, can be degraded by autophagy, thus this process was proposed as a mechanism utilized by senescent cells to remove damaged genomic material (Ivanov et al. 2013; Dou et al. 2015; 2017). The loss of chromatin would impact on the chromatin landscape, gene expression and long-term phenotype of senescent cells (O'Sullivan et al. 2010; Ivanov et al. 2013). However, emerging evidence indicates that leaked cytoplasmic DNA in turn can also trigger a discrete aspect of senescence, the non-autonomous activity, via activation of the cGAS/STING pathway which is discussed further in the next section.

### **Non-autonomous effectors**

In addition to their essential feature (the persistency of cell cycle arrest), senescent cells are actively engaged in communication with other cells and the extracellular matrix (ECM) within the tissue microenvironment. Several modes of senescence-associated cell-cell communication have been shown including the SASP, cell fusion (Chuprin et al. 2013), cytoplasmic bridges (Biran et al. 2015), cell-cell contact (Nelson et al. 2012; Hoare et al. 2016; Parry et al. 2018; Ito et al. 2017) and secreted extracellular vesicles (Takasugi et al. 2017).

The SASP is the best characterised of these mechanisms (Kuilman and Peeper 2009; Malaquin et al. 2016), and the non-autonomous 'functionality' of senescent cells has been mostly attributed to the SASP. The fact that senescent cells secrete soluble factors was long known and expression of some factors, such as MMP3 and PAI1, had been used as markers of senescence (Krtolica et al. 2001; Parrinello et al. 2005; Kortlever et al. 2006). Initially, the SASP was collectively assumed to have tumor promoting effects (Campisi and d'Adda di Fagagna 2007), because senescent fibroblasts promote the tumorigenesis of co-existing premalignant epithelial cells (Krtolica et al. 2001). The SASP has since been demonstrated as a part of senescence program, itself with diverse downstream effects. For example, PAI1 was shown to be a downstream effector of the p53 pathway during replicative senescence, which contributes to cell cycle arrest; while IL6 and IL8, an inflammatory cytokine and chemokine respectively, reinforce senescence arrest through paracrine activities (Kortlever et al. 2006; Kuilman et al. 2008; Acosta et al. 2008; Wajapeyee et al. 2008). Increasing evidence has revealed that the SASP is a highly dynamic process, associated with diverse components, regulatory mechanisms, and functionalities.

#### *Inflammatory SASP and senescence surveillance*

Among the diverse components, the best characterised factors of the SASP are of the pro-inflammatory type, represented by IL1, IL6 and IL8 among others. Expression of these inflammatory SASP components is controlled by diverse factors, such as epigenetic and chromatin regulators, the translation machinery, and signaling molecules (Tasdemir et al. 2016; Takahashi et al. 2012; Freund et al. 2011; Laberge et al. 2015; Herranz et al. 2015; Rodier et al. 2009; Hoare et al. 2016; Capell et al.

2016; Aird et al. 2016; Dou et al. 2017). The central mode of SASP regulation is a hierarchical model, where the genes encoding the proximal inflammatory cytokines, such as IL1 and IL6, are induced by the inflammatory transcription factors, NF $\kappa$ B and C/EBP $\beta$ , cooperatively with co-factors, such as BRD4 (Tasdemir et al. 2016). Importantly, these inflammatory cytokines in turn reinforce the activity of those transcription factors through a positive feedback loop, thus the signaling is locally amplified (Kuilman et al. 2008; Orjalo et al. 2009; Acosta et al. 2013; Pérez-Mancera et al. 2014).

But how is such a signaling cascade subsequently resolved? The major outcome of the inflammatory SASP *in vivo* is not just senescence reinforcement, but also activation of an immune reaction (Fig. 1). The first *in vivo* evidence for this was derived from a p53-deficient liver cancer model, where cancer cells express a tetracycline-regulable p53 RNAi (Xue et al. 2007). Restoration of endogenous p53 after the cancer was established led to the induction of senescence and tumor regression, which was not due to apoptosis but immune clearance of senescent liver tumor cells. Although this experiment was conducted in athymic nude mice, which are immuno-compromised, their innate immune system is intact and sufficient for the clearance of senescent cells in this particular context. A follow-up study in mice lacking T and B cells reinforced the sufficiency of innate immune cells, in this case NK cells, in mediating tumor suppressive clearance of senescent cells (Iannello et al. 2013).

Subsequently, a similar elimination of senescent cells by innate immune cells has been shown in other models, such as chronic liver damage in mice (Krizhanovsky et

al. 2008; Lujambio et al. 2013). Krizhanovsky et al. showed that the liver damaging agent, CCl<sub>4</sub>, induces senescence in the liver, but those senescent cells are mainly hepatic stellate cells (HSCs). HSCs are fibroblast-like cells, which are normally in a quiescent state but become active and proliferative, followed by the development of senescence. Through the SASP, senescent HSCs can be eliminated by innate immune cells, including natural killer cells and tumor inhibiting M1-state macrophages (Krizhanovsky et al. 2008; Lujambio et al. 2013). Interestingly, p53-deficient HSCs bypass senescence and secrete factors that stimulate macrophages into a tumor promoting M2 state, suggesting a non-autonomous tumor suppressor activity for p53 (Lujambio et al. 2013). This study also highlights that the SASP derived from senescent stromal cells has a profound impact on the tumor microenvironment.

These 'TIS' (i.e. restoration of endogenous p53) or damage-induced senescence studies established the role of innate immunity in senescence clearance, but a role for adaptive immune components in senescence surveillance was shown in a liver OIS model. As shown by Kang et al. (Kang et al. 2011), ectopic expression of oncogenic NRas induces senescence in hepatocytes in mice and those OIS hepatocytes are eliminated primarily by macrophages, but this process requires activation of mutant NRas specific CD4<sup>+</sup> T cells. Indeed, immune clearance of OIS hepatocytes is diminished when the experiment was conducted in immune-compromised mice, which eventually developed liver cancer (Kang et al. 2011). Interestingly, major histocompatibility complex class II (MHCII) is typically expressed by professional antigen presenting cells (APCs) but hepatocytes appear to express MHCII to directly present antigens (Herke et al. 2003). Indeed, a fraction of the OIS hepatocytes appear to express higher level of MHCII, which, although not sufficient (i.e. professional APCs

are necessary), is required for senescence surveillance (Kang et al. 2011). Similar upregulation of MHCII was reported in melanocytic OIS models. In contrast to the liver model, however, MHCII on OIS melanocytes appears to be sufficient for T cell activation (van Tuyn et al. 2017). These studies suggest that the senescence surveillance involves multi-levelled and distinct aspects of the immune system depending on the context, such as the senescence trigger and cell type.

#### *Other SASP functionality*

The functionality of the SASP is not limited to mediating immune clearance of senescent cells from the tissue. It also plays a role in the maintenance of tissue integrity. As mentioned above, upon liver damage, quiescent HSCs become activated and proliferate before developing senescence (Krizhanovsky et al. 2008). Activated HSCs produce ECM components, contributing to liver fibrosis. Importantly, the SASP components of senescent HSCs contains ECM degradation enzymes, thus countering the excessive fibrosis. Thus, tissue repair and integrity are controlled through a timely switch from a fibrogenic to a fibrolytic secretory program, and this switch is coupled with the eventual elimination of senescent cells by immune cells. A similar role for senescence in tissue repair and wound healing has been shown in other tissue damage models (liver (Kong et al. 2012; Kim et al. 2013; Borkham-Kamphorst et al. 2014), kidney (Wolstein et al. 2010), skin (Jun and Lau 2010b; Pitiyage et al. 2011; Demaria et al. 2014), and heart (Zhu et al. 2013)). Notably, Krizhanovsky et al. also showed in the liver model (Krizhanovsky et al. 2008) that persistence of senescent HSCs due to the failure of immune-mediated elimination of senescent HSCs rather promotes liver fibrosis, reinforcing the idea of a senescence life cycle. Association

between fibrogenic senescence and chronic tissue damage/inflammation has also been reported in an idiopathic pulmonary fibrosis mouse model (Schafer et al. 2017).

More recently, a progressive switch of secretory factors has also been shown in the classical senescence model in culture, where NOTCH signaling is transiently activated during an early phase of senescence triggered by oncogenic HRas or DNA damage (Hoare et al. 2016). The NOTCH phase is correlated with a distinct secretome, representing TGF $\beta$  and other fibrogenic factors, and subsequent downregulation of NOTCH signaling is required for switching to the typical inflammatory and fibrolytic SASP in the late phase of senescence. This is highly reminiscent of the case of HSC senescence (Krizhanovsky et al. 2008), but it remains to be elucidated whether or not Notch signaling is activated during the progressive development of HSC senescence. Mechanistically, it was shown that NOTCH signaling negatively regulates the expression of primary inflammatory cytokine production through suppressing the activity of the transcription factor C/EBP $\beta$ . Consistently, in the liver OIS model described above, the inhibition of NOTCH signaling in NRas-expressing hepatocytes facilitates their elimination (Ito et al. 2017).

A recent addition to the SASP functionality includes its role in cellular reprogramming (reviewed in (Taguchi and Yamada 2017)). Earlier studies had shown that the senescence machinery autonomously serves as a barrier to reprogramming into induced pluripotent stem cells (iPSCs) in response to expression of the four Yamanaka factors (Oct3/4, Sox2, Klf4, and c-Myc) (Krizhanovsky and Lowe 2009; Li et al. 2009; Banito et al. 2009; Hong et al. 2009; Kawamura et al. 2009; Marión et al. 2009; Utikal

et al. 2009). However, using mice expressing those Yamanaka factors, recent studies have shown that senescent cells non-autonomously facilitate the reprogramming of surrounding cells through an NF $\kappa$ B-driven secretome, particularly IL6 (Mosteiro et al. 2016; Chiche et al. 2017). Consistently, Ritschka et al. showed that a transient or low-level, but not prolonged, exposure to the OIS SASP promotes cellular plasticity and regenerative capacity in mouse skin keratinocytes (Ritschka et al. 2017). It has been suggested that senescence arrest in tissue stem cell or progenitor cells reduces regenerative capacity in some tissues (Sharpless and DePinho 2007). In contrast to this autonomous effect of senescence, much like the case of cellular reprogramming to iPSC, senescent cells appear to non-autonomously contribute to maintaining the tissue stem cell niche.

The physiological roles of senescent cells in facilitating tissue repair in response to injury relies on their clearance after the cessation of the wound healing processes, as accumulation of senescent cells conversely favour the pathogenesis of aging and age-related disorders. Tumors have long been dubbed as “wounds that do not heal” (Dvorak 1986; Byun and Gardner 2013; Dvorak 2015), due to similarities between the phases of wound healing and that of tumor stroma formation. Through the SASP (derived from either tumorous or stromal senescent cells), the chronic presence of senescent cells within a tumor microenvironment would maintain a chronically inflamed microenvironment that is inherently tumorigenic (Lecot et al. 2016; Baker et al. 2017). The SASP also modulates other key hallmarks of cancers through locally facilitating vascularization (Coppé et al. 2006), epithelial-mesenchymal transition (Coppé et al. 2008; Kuilman et al. 2008), tumor invasion (Kim et al. 2017; He et al.

2018) and cellular plasticity (Ritschka et al. 2017). Disruption of the senescence life cycle may create a tumorigenic microenvironment collectively through these SASP functionalities (Fig. 1).

### *Upstream regulators of SASP*

The inflammatory SASP appears to be controlled through multiple effectors. For example, the SASP is typically promoted by a persistent DNA damage response (Rodier et al. 2009; Ciccia and Elledge 2010). However how the inflammatory cascade is initiated had not been clear until a recent series of publications showed that the cGAS-STING cytosolic DNA sensing pathway, described in the previous section, is a critical upstream event to trigger the SASP (Dou et al. 2017; Glück et al. 2017; Takahashi et al. 2018; Li and Chen 2018). Evolved as a defence response to viral and microbial infections, this pathway promotes inherent anti-tumor immunity (Deng et al. 2014; Woo et al. 2014; Bose 2017). The cGAS-STING pathway, an intrinsic tumor suppressor, is deregulated in carcinomas (Xia et al. 2016) and low levels of cGAS or STING are correlated with poor prognosis in some cancer types (Song et al. 2017; Yang et al. 2017a). Interestingly, the same pathway appears to be provoked during senescence, sensing CCFs (Ivanov et al. 2013; Dou et al. 2015).

The DNA sensor cGAS, upon binding to DNA, produces the second messenger cGAMP, which activates STING. This typically leads to the engagement of two canonical downstream pathways: A type I interferon (IFN) response mediated by IRF3 and an inflammatory response through NFkB activation (Abe and Barber 2014; Barber 2015). However, type I IFNs are not necessarily overrepresented in the SASP,



depending on the cell type (Dou et al. 2017). The researchers proposed that this is in part due to the activation of p38MAPK (another positive regulator of the SASP (Freund et al. 2011)), which can inhibit STING-mediated IFN induction (Dou et al. 2017). Thus, the cGAS-STING pathway is critical in the activation of the NFkB-driven SASP. Consistently, the loss of this pathway results in reduced senescence surveillance and increased tumorigenesis in the liver OIS model (Glück et al. 2017; Dou et al. 2017). As with the inflammatory SASP, it is conceivable that prolonged activation of cGAS-STING can also promote tumorigenesis and metastasis, possibly through chronic inflammation (Li and Chen 2018). Indeed, in an obesity-induced liver cancer model, the SASP-derived from senescent HSCs promotes liver cancer (Yoshimoto et al. 2013) and this effect was diminished in Sting knockout mice (Dou et al. 2017).

### *Autophagy*

It has been shown that the SASP is also controlled through macroautophagy (simply autophagy hereafter), another effector of senescence (Hoare et al. 2011). Autophagy, an evolutionarily conserved catabolic machinery, involves the formation of autophagosomes and their fusion to lysosomes to form autolysosomes, where encircled macromolecules or even organelles, are digested by lysosomal enzymes (Shen and Mizushima 2014). The bulk degradation of damaged macromolecules facilitates their turn-over and the degradation products can be an alternative energy source. Thus, basal autophagy is important for the quality control of cellular components and energy homeostasis. In addition to its basal activity, autophagy can also be acutely activated by not only metabolic (e.g. starvation) but also cytotoxic (e.g. DNA damage and oncogenic) stress. It is conceivable that stress-induced autophagy

contributes to the degradation of damaged cellular components, but its long-term functional relevance is not entirely clear.

One of the best-known markers of senescence is an increase in senescence-associated  $\beta$ -galactosidase activity (SA- $\beta$ -gal), which is derived from a lysosomal enzyme (Lee et al. 2006), and earlier studies have suggested that autophagy is activated during senescence and SA- $\beta$ -gal reflects increased lysosomal mass at least in some contexts (Gerland et al. 2003; Narita et al. 2011; Kurz et al. 2000). Functionally, it has been suggested that autophagy contributes to the senescence phenotype in part through modulating the SASP (Patschan et al. 2008; Young et al. 2009). Interestingly, prolonged activation of autophagic protein degradation was shown to activate the anabolic counterpart of autophagy, mTOR on the surface of (auto)lysosomes through degradation products (i.e. amino acids) (Yu et al. 2010; Efeyan et al. 2012). Increased lysosomal biogenesis and compartmentalization during senescence have been shown in several models (Young et al. 2009; Narita et al. 2011; Dörr et al. 2013). It was proposed that prolonged activation of autophagy leads to the simultaneous activation of mTOR (which is typically regulated in the opposite direction to autophagy), which facilitates protein synthesis (Narita et al. 2011). How mTOR signaling specifically activates the SASP was not clear, but recent studies have proposed multiple mechanisms for this through modulating translation and the stabilization of mRNA molecules encoding SASP components (Herranz et al. 2015; Laberge et al. 2015; Tomimatsu and Narita 2015) (Fig. 3).

The positive regulation of senescence by autophagy was counter-intuitive, considering the role of autophagy in cellular quality and fitness checks, although it is possible that

increased autophagy activity serves as a pro-survival factor in senescent cells, which are known to be resistant to the cell death machinery (Dörr et al. 2013). The relevance of autophagy in senescence appears to be highly context dependent (Kwon et al. 2017). It is probably important to consider the difference between basal and stress-induced autophagy, a lack of the former would lead to the accumulation of damaged macromolecules (such as p62-containing aggresomes) and damaged organelles (such as dysfunctional mitochondria). Thus, it is conceivable that loss of basal autophagy alone promotes senescence through cellular damage (Kwon et al. 2017). For instance, a role of basal autophagy in preventing senescence of muscle satellite cells thus maintaining stemness has previously been reported (García-Prat et al. 2016). However, in addition to this autonomous activity of autophagy, autophagy affects a non-autonomous aspect of senescence (i.e. the SASP) in the context of chronic stress-induced autophagy. In addition, autophagy also contributes to other senescence effectors, such as (epi)genetic modulation through Lamin B1 degradation (Dou et al. 2015; Lenain et al. 2015). As mentioned earlier in this review, autophagy-mediated degradation of Lamin B1 also leads to the formation of CCFs, which could activate the cGAS-STING-SASP axis (Fig. 3).

Another layer of regulation of the SASP by autophagy was recently identified. Autophagy is generally considered to be a non-selective bulk degradation machinery, but it involves some level of selectivity through autophagy receptor proteins, such as p62/SQSTM1 (Komatsu and Ichimura 2010). Kang et al. have shown that the transcription factor GATA4 positively controls the NFkB-driven SASP (Kang et al. 2015). They found that GATA4 is a substrate of p62-mediated selective autophagy, which is, in contrast to general autophagy, inhibited during senescence, indicating that

selective autophagy can negatively regulate the inflammatory SASP. Thus overall, autophagy appears to affect the SASP at multiple levels in a non-unidirectional manner (Fig. 3).

## **Conclusion**

Senescence blocks the proliferation of unnecessary or unwanted cells in both physiological and pathological contexts. Due to this autonomous aspect, senescence has been proposed to be a therapeutic goal of cancer therapy. While the senescence arrest can be an intrinsic tumor suppressor mechanism, particularly at the early stages of tumorigenesis, this view turns out to be too simplistic. Senescent cells are metabolically active and have a profound impact, often deleterious in the long-term, on their neighbours through their non-autonomous activities. Interestingly, however, these autonomous and non-autonomous programs appear to be mechanistically linked. While the causal relevance of epigenetic changes during senescence remains elusive, the distinct epigenetic landscape in senescence has been correlated with the SASP (Aird et al. 2016; Tasdemir et al. 2016; Parry et al. 2018). Loss of Lamin B1 appears to orchestrate high-order chromatin structural alterations, genomic instability, and the formation of CCFs, which in turn triggers the SASP (Sadaie et al. 2013; Shah et al. 2013; Dou et al. 2015; 2017). Autophagy, an autonomous effector by nature, also affects the SASP (Fig. 3). Further understanding of how the 'irreversibility' of cell cycle exit is mechanistically and functionally coupled with non-autonomous effectors of senescence will be of great interest. Georgilis et al. have recently identified a number of druggable targets, the inhibition of which can induce senescence without the inflammatory SASP (Georgilis et al. 2018). Such de-coupling between cell cycle

arrest and the SASP might provide a promising therapeutic modality for pro-senescence cancer therapy, with reduced complications from the SASP.

Alternatively, senescence itself has recently been proposed to be a therapeutic target, rather than a goal. After the seminal work by van Deursen and colleagues, showing that the genetic ablation of senescent cells provides beneficial effects on health lifespan in mice (Baker et al. 2011), a number of small molecules that selectively kill senescent cells (collectively called senolytics) have been developed (Yosef et al. 2016; Zhu et al. 2015; Baar et al. 2017; Kirkland and Tchkonja 2017; Zhu et al. 2016). Mostly, these reagents have been used (Demaria et al. 2017) in the context of aging and tissue damage (Xu et al. 2018). In the cancer context, it was shown that killing stromal senescent cells induced by chemotherapy reduces the associated side effects, such as cardiac and liver dysfunction, and general fatigue (Demaria et al. 2017; Baar et al. 2017).

However, considering the potential adverse effects of the SASP derived from TIS, administering 'senolytics adjuvants' in conjunction with pro-senescence therapy in cancer might be a promising approach. In fact, it was shown that targeting the metabolic pathways increased in senescent cells (e.g. autophagy or glucose utilization) in TIS lymphoma cells leads to the selective killing of TIS cells and an improved treatment outcome (Dörr et al. 2013). A more recent study also suggests that selective targeting of TIS cells using sugar-coated (thus sensitive to  $\beta$ -galactosidase) nanoparticles encapsulating cytotoxic drugs is beneficial in xenograft models (Muñoz-Espín et al. 2018).

Senescence, or even the SASP, is mostly tumor suppressive in the early stages, particularly when the senescent cells are eliminated by the immune system. This intrinsic phase might also be exploited for tumor therapy (Fig. 1). For example, it was shown that genetic inhibition of Notch signaling in the hepatocyte OIS model facilitates senescence surveillance (Hoare et al. 2016). We proposed that OIS might be most relevant at the stage of oncogenic ‘amplification’ (genetically or functionally) after somatic mutations, which are often found in normal tissues (Fig. 2) (Risques and Kennedy 2018). Selective targeting of cells at this stage might also be beneficial for tumor therapy or prevention.

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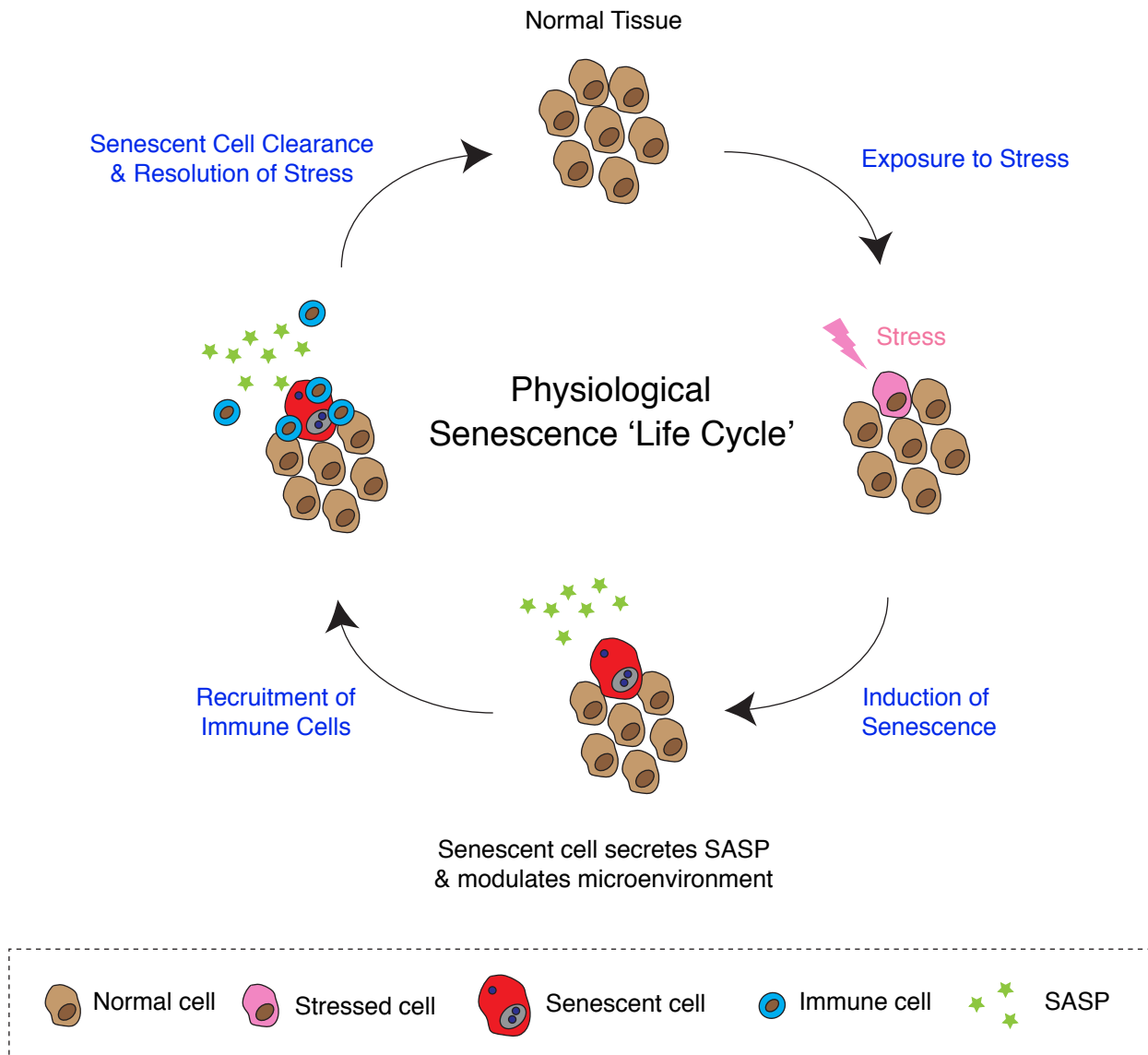
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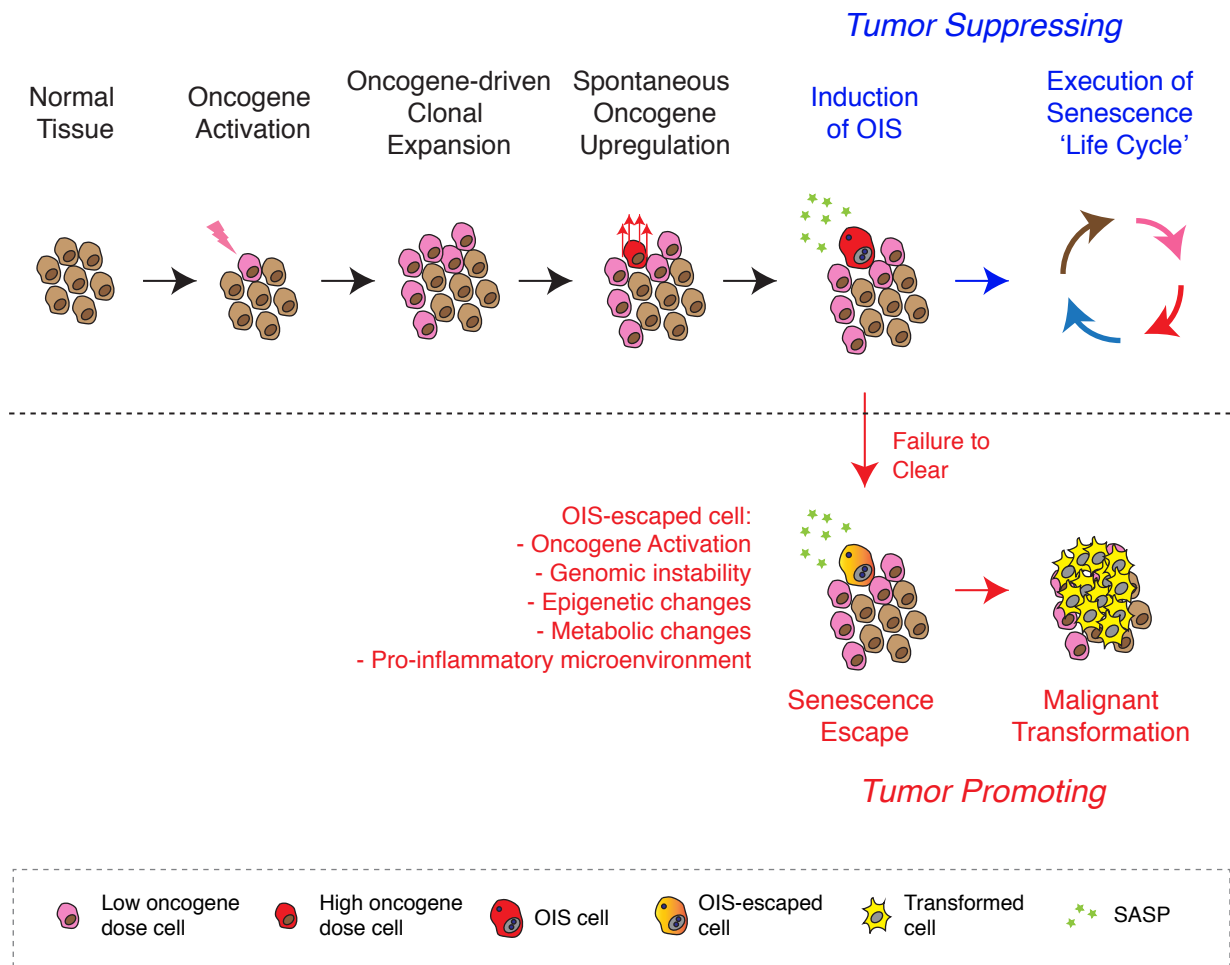


**Figure 1. Senescence 'life cycle'**

Phases of the 'senescence life cycle': Cells undergo senescence in response to stress in normal (non-cancerous) tissues. As part of the senescence programme, the secretome is modified to include upregulation of pro-inflammatory cytokines and chemokines which modulate the tissue microenvironment. This recruits immune cells and facilitates clearance of senescent cells, mediating resolution and restoring tissue homeostasis. Deviation from this fail-safe mechanism can instead lead to age-related pathology or cancer. In some cases, cells become activated / proliferative before senescence establishment, and senescence signaling can be locally amplified through non-autonomous activities (these points are not reflected in the figure for simplicity).



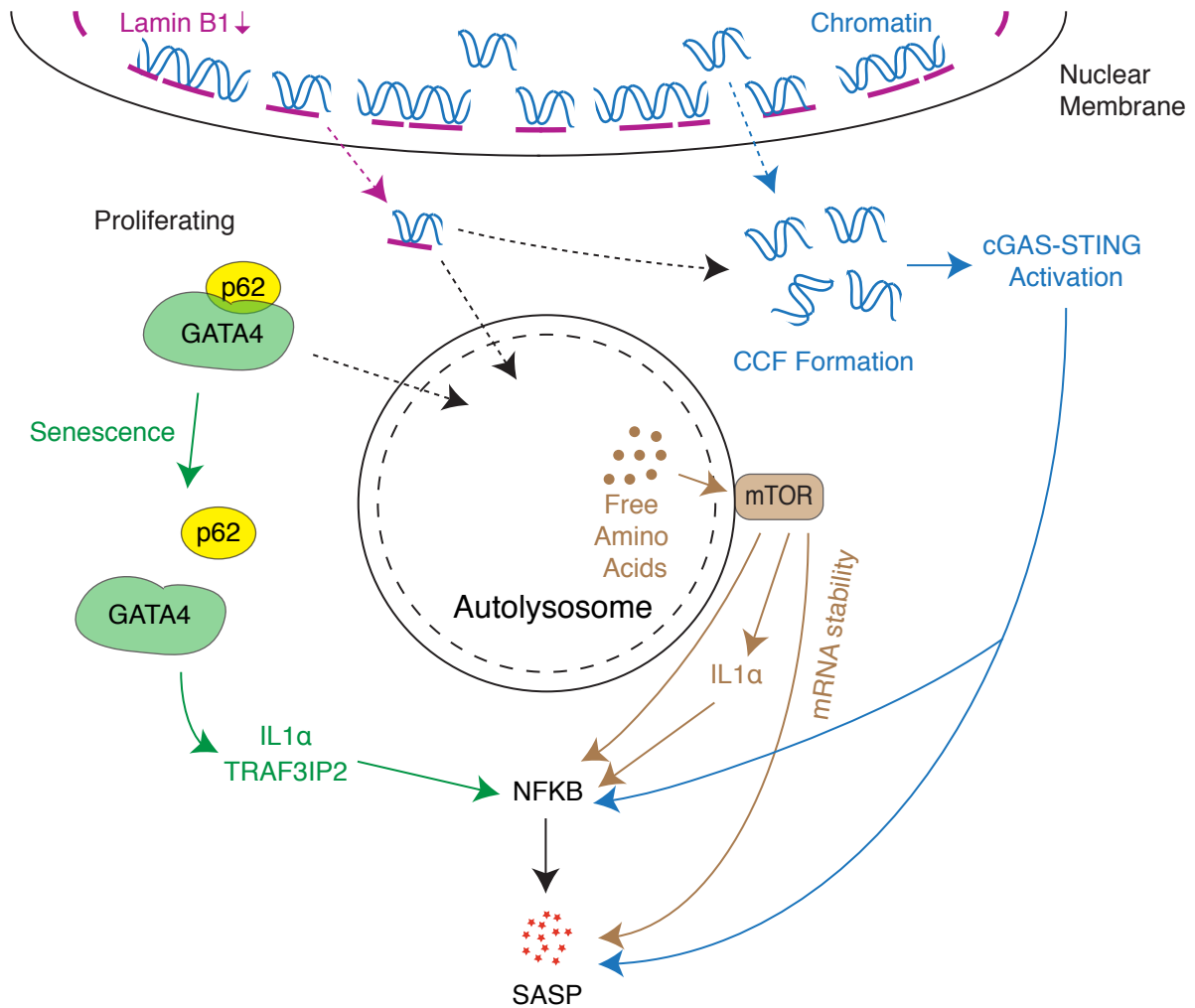
## GENESDEV/2018/320937\_Chan\_Figure 2



**Figure 2. OIS as a model of spontaneous upregulation of somatically mutated oncogenic signaling**

Using oncogenic Ras as an example, an age-dependent increase of somatic mutation of oncogenes and their clonal expansion are common, but high-levels of oncogenic signaling is necessary for both OIS and full malignant transformation. Typically, spontaneous upregulation of oncogenic signaling (to the levels sufficient for malignancy) triggers the OIS program, which is tumor suppressive as long as the 'senescence life cycle' is executed to completion. Failure to clear OIS cells can conversely be tumor promoting, as these cells are at risk of senescence escape having acquired tumor-facilitating cellular changes as well as shaped a pro-tumorigenic microenvironment.

## GENESDEV/2018/320937\_Chan\_Figure 3



**Figure 3. Multiple non-unidirectional levels through which autophagy-related processes in senescence affect regulation of SASP genes, often via NFκB activation.**

Activation of macroautophagy as an effector of senescence and spatial coupling of mTOR with autolysosomes leads to mTOR activation, which has been proposed to modulate SASP expression through multiple mechanisms (brown arrows). Autophagy-mediated degradation of Lamin B1 also promotes CCF formation, which upregulates SASP genes through the cGAS-STING pathway. This has been suggested to occur both by nuclear membrane blebbing (purple arrows), which shuttles LADs to the cytoplasm, as well as loss of nuclear envelope integrity allowing escape of chromatin fragments (blue arrows). However, the activation of general autophagy during senescence is accompanied by an inhibition of p62-mediated selective autophagy, allowing stabilisation of the GATA4 transcription factor, which regulates SASP genes via NFκB (green arrows). Dotted arrows denote physical movement, solid arrows denote signaling.