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The Senescence-Associated Secretory Phenotype: Critical Effector in Skin Cancer and Aging

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Abstract

Cellular senescence, a state of stable cell cycle arrest in response to cellular stress, is an indispensable mechanism to counter tumorigenesis by halting the proliferation of damaged cells. However, through the secretion of an array of diverse cytokines, chemokines, growth factors, and proteases known as the senescence-associated secretory phenotype (SASP), senescent cells can paradoxically promote carcinogenesis. Consistent with this, removal of senescent cells delays the onset of cancer and prolongs lifespan in vivo, potentially in part through SASP reduction. In this review, we consider the evidence for the SASP and "SASP-like" inflammation in driving skin carcinogenesis, emphasizing how further understanding of both the roles and mechanisms of SASP expression may offer new targets for skin cancer prevention and therapy.

INTRODUCTION

In the 1960s, Leonard Hayflick and Paul Moorhead made a monumental discovery: normal cells have a finite replicative potential in vitro, regardless of culture conditions (Hayflick, 1965). This stable arrest of the cell cycle has been termed cellular senescence, and is believed to be a primary defense against the unlimited cellular proliferation that drives cancer (Mooi and Peeper, 2006). In the decades since, we have learned that senescence is not only caused by this progressive telomere shortening-induced replicative threshold but also by a diverse array of cellular stressors ranging from activated oncogenes and ultraviolet radiation (UVR) to DNA-damaging therapies, oxidative stress, and the depletion of deoxyribonucleotide pools (Mannava, 2013; van Deursen, 2014). For instance, an activated $BRAF^{V600E}$ oncogene can drive the initial proliferation of human melanocytes, which then ultimately growth arrest to form a senescent nevus (Michaloglou et al., 2005). However, senescent cells can escape this cell cycle arrest to form cancers such as melanoma (Figure 1a) (Liu and Sharpless, 2012).

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Beyond tumor suppression and cancer, senescent cells have been implicated in critical physiological processes including development, wound healing, and normal aging (Childs et al., 2015). Indeed, the number of senescent cells increases with aging in human tissues, including in both the skin dermis and epidermis (Dimri et al., 1995; Munoz-Espin and Serrano, 2014; Ressler et al., 2006). How senescent cells can have such pleiotropic effects is explained by their ability to secrete an array of inflammatory cytokines, chemokines, growth factors, and proteases known as the senescence-associated secretory phenotype (SASP) (Campisi, 2013; Coppe et al., 2010; Neves et al., 2015). These factors include such well-known cellular mediators as IL1α, IL1β, IL6, IL8, and matrix metalloproteinases (MMP1 and MMP3) (Figure 1b, demonstrating IL6 within a nevus, and Figure 2). Although the role of senescence in aging and wound healing was recently reviewed in the JID (Demaria et al., 2015), here we focus on the SASP, its regulation, and its role in the development and progression of cancer, with particular attention paid to the evidence from melanoma and nonmelanoma skin cancers. As in many other fields of research, numerous mechanistic findings in the senescence field have been initially uncovered in primary human fibroblasts. Although keratinocytes and melanocytes have typically displayed similar responses and comparable results when subjected to identical experimental conditions, this caveat must be kept in mind when interpreting results. Further, as cancer-associated fibroblasts are frequently pivotal players in driving carcinogenesis, consideration of cell-type specificity is critical when developing a unified understanding of the role of the SASP in skin carcinogenesis (Gascard and Tlsty, 2016). Thus, in this review, we attempt to note and clarify the cell types employed whenever feasible.

Regulation of the SASP

Although the SASP is associated with senescence, it can be detached from the actual growth arrest of senescence. For instance, in primary human fibroblasts and mammary epithelial cells, overexpression of the cyclin-dependent kinase inhibitor p16INK4a (*CDKN2A*) is sufficient to induce senescence growth arrest (Coppe et al., 2011). However, without any precipitating DNA damage, there is no SASP in these studies. In contrast, DNA damage leads to a SASP independent of p16INK4a levels (Coppe et al., 2011). These data are in agreement with other studies demonstrating the requirement of an activated ataxia telangiectasia mutated (ATM)-mediated DNA-damage response (DDR) for SASP expression (Rodier et al., 2009) (Figure 3). In a seminal study employing human foreskin fibroblasts and mammary epithelial cells, Di Micco et al. (2006) demonstrated that expression of oncogenic HRasV12 leads to oncogene-induced senescence in a DDR and DNA replication-dependent manner, as cells are unable to induce the DDR when DNA replication is inhibited. Rodier et al. (2009) then later showed that this DDR-SASP link required ATM, but not other DDR regulators, ATR or p53. Mechanistically, ATM is able to couple replication stress to both DDR activation and metabolic reprogramming during senescence (Aird et al., 2015). Despite this, an exception to this DDR-SASP connection was observed during embryonic developmental senescence in mesonephric epithelial cells: although overall displaying a unique gene expression program from damage-induced senescence, embryonic senescence was noted to engage the transforming growth factor-β pathway in the absence of markers of DNA damage (Muñoz-Espín et al., 2013). Collectively, these data demonstrate that although the SASP is typically a response to DNA damage rather than cellular proliferation arrest per

se, DNA damage may not be absolutely required in the case of developmentally programmed senescence.

The DDR leads to increased transcription of cytokines such as IL1α, IL1β, IL6, IL8, proteases MMP1 and MMP3, and growth factors including fibroblast growth factor and hepatocyte growth factor among others. In studies performed in both foreskin fibroblasts and mammary epithelial cells, IL1α has been shown to be an upstream regulator of other SASP factors during oncogene-induced senescence, including IL6, IL8, vascular endothelial growth factor, and transforming growth factor-β (Acosta et al., 2013; Orjalo et al., 2009). Critical to the transcription of the SASP is the phosphorylation of the transcription factor NF-κB (Freund et al., 2010) (Figure 3). On senescence or other DNA-damaging stimuli in human embryonic fibroblasts, Chien et al. (2011) demonstrated that the phosphorylated NF-κB p65/RelA subunit translocates to the nucleus where it binds the promoters of several SASP genes, thus regulating their induction during senescence or DDR. For this reason, NF-κB has often been termed the “master regulator” of the SASP, as its activation is critical for SASP expression. In addition to NF-κB, other transcription factors have also been implicated in SASP activation. CCAAT/enhancer binding protein β is a transcription factor regulated by the mitogen-activated protein kinase (MAPK) pathway and has been shown to be necessary for oncogene-induced senescence in mouse embryonic fibroblasts (Sebastian et al., 2005). Along with NF-κB, CCAAT/enhancer binding protein β activation is increased during senescence along with its mRNA levels in both human fibroblasts and melanocytes (Kuilman et al., 2008). Knocking down this factor using shRNA leads to decreased levels of CXCR2 ligands, suggesting that NF-κB and CCAAT/enhancer binding protein β work together in regulating the SASP (Acosta et al., 2008) (Figure 3). Finally, recent work in human foreskin fibroblasts has established GATA4 nuclear stabilization as an inducer of IL1α and upstream activator of NF-κB (Kang et al., 2015). GATA4, which is normally degraded by p62-mediated autophagy, is stabilized in the nucleus during senescence, where it can induce IL1α to increase the SASP through NF-κB activation via the ATM-mediated DDR pathway (Kang et al., 2015). Thus, depending on the precise cellular context, several transcription factors are active in the nucleus that can all play a part in SASP regulation (Figure 3).

Beyond the canonical DDR pathway, initiated by ATM phosphorylation, in different contexts, other kinases have been shown to play a role in SASP regulation. For example, the p38MAPK pathway has been demonstrated to be sufficient to induce the SASP in neonatal skin fibroblasts (Freund et al., 2011) (Figure 3). Upregulation of p38MAPK results in the increased activation of NF-κB, consistent with known processes that connect Ras and MAPK signaling to the activation of NF-κB (Freund et al., 2011; Norris and Baldwin, 1999). In addition, other work has also identified protein kinase D1 (PKD1), a Ca^{+2}/calmodulin-dependent kinase, as a major regulator of the SASP in human fibroblasts through NF-κB (Wang et al., 2014). In these studies, PKD1 levels directly correlated with those of certain SASP factors, and PKD1 overexpression decreased levels of the NF-κB inhibitor, IκB, through phosphorylation of the IKK complex, suggesting that PKD1 is able to affect NF-κB activity during senescence and regulate the SASP (Wang et al., 2014) (Figure 3).

The presence of the SASP indicates that even after cell cycle arrest, cell metabolism is still active, which permits the release of cytokines. This knowledge led to studies involving the mammalian target of rapamycin (mTOR) pathway, a major regulator of cellular metabolism. mTOR is a protein kinase that has been shown to increase protein translation and promote cellular senescence (Xu et al., 2014) (Figure 3). mTOR is a component of two complexes, mTORC1 and mTORC2, although only the former has been shown to have a major effect in senescence. Consistent with these findings, the drug rapamycin inhibits mTORC1, which delays both replicative senescence and oncogene-induced senescence in human foreskin fibroblasts (Kolesnichenko et al., 2012). This delay of senescence in vitro has been correlated to increased lifespan in vivo in yeast, *Caenorhabditis elegans*, Drosophila, and mice (Harrison et al., 2009; Kaeberlein et al., 2005; Kapahi et al., 2004; Vellai et al., 2003). In addition, recent studies have found that rapamycin inhibition of mTORC1 also reduces IL1α translation, leading to reduced NF-κB activity and decreased SASP expression, while maintaining the senescence-associated growth arrest in multiple fibroblast and epithelial cell lines (Laberge et al., 2015). Further, Herranz et al. (2015) recently demonstrated that mTOR regulation of SASP occurs through 4 enhancer binding protein-mediated MAPK-activated protein kinase 2 translation and resultant ZFP36L1 phosphorylation, elucidating another mechanism through which rapamycin treatment is able to regulate SASP expression. In line with these findings, the type 2 diabetes medication, metformin, which has been shown to inhibit mTOR activity, also decreases SASP expression in human fibroblasts (Moiseeva et al., 2013).

Given that the SASP is primarily regulated at the transcriptional level, it is not surprising that recent studies have begun to uncover roles for epigenetic regulators in SASP expression as well (Figure 3). It was initially established that senescent cells could display chromatin alterations through the observation of senescence-associated heterochromatic foci that can be seen in fibroblasts on senescence induction (Narita et al., 2003). More recently, histone deacetylase inhibitor treatment has been noted to induce SASP expression in a variety of human fibroblast cell lines (Pazolli et al., 2012). Further, another deacetylase with well-established connections to aging, Sirt1, has been shown to bind SASP promoters. In the setting of DNA damage, it detaches and permits histone acetylation and induction of SASP genes in human skin fibroblasts (Hayakawa et al., 2015). The DDR also leads to the ubiquitination of key methyltransferases involved in gene silencing in the regions around SASP promoters, thus allowing enhanced transcription of SASP genes in human dermal fibroblasts (Takahashi et al., 2012). One methyltransferase in particular, the H3K4me3 methyltransferase and oncoprotein, mixed-lineage leukemia 1, is a critical component of DDR activity during the S-phase checkpoint (Liu et al., 2010). In line with this, recent work by Capell et al. (2016) has demonstrated that mixed-lineage leukemia 1 inhibition represses SASP expression in both human fibroblasts and melanocytes, while simultaneously maintaining the senescent state, thus providing a potential target for abrogating the deleterious proinflammatory phenotype of the SASP while maintaining tumor-suppressive cell cycle arrest. Finally, in addition to histone modifying enzymes, very recently the bromodomain protein, Brd4, was shown to be recruited to SASP gene enhancers and critical for their expression in human fibroblasts. Similar to targeting mixed-lineage leukemia 1

epigenetic activity, inhibition of Brd4 with small molecule BET inhibitors could prevent the SASP in these studies (Tasdemir et al., 2016).

Effects of the SASP

Extensive research into the SASP has resulted in a paradox: the set of cytokines released during DNA damage and senescence can have both beneficial and detrimental effects. As noted above, p21-mediated senescence has now been shown to play a critical role in embryonic development, with secreted SASP-like factors such as transforming growth factor-β playing a critical role (Munoz-Espin et al., 2013; Storer et al., 2013). Further, the SASP has been shown to induce senescence of surrounding normal fibroblasts through a paracrine mechanism, resulting in the increased clearance of potentially tumorigenic cells via immune infiltration (Kuilman et al., 2008; Neves et al., 2015). In melanocytes, a secreted factor, IGFBP7, has been shown to be essential for BRAF-induced senescence (Wajapeyee et al., 2008). Finally, SASP cytokines have been shown to play a pivotal role in wound healing. Demaria et al. (2014) demonstrated that the accumulation of senescent cells and their associated secretion of platelet-derived growth factor AA near injury sites induced wound closure through increased myofibroblast differentiation in vivo.

In contrast, the SASP has also been associated with a host of deleterious effects, ranging from skin aging (through MMP1-mediated collagen breakdown) and osteoarthritis to cardiovascular disease and diabetes (Demaria et al., 2015; Fyhrquist et al., 2013; Greene and Loeser, 2015; Munoz-Espin and Serrano, 2014). Perhaps most counterintuitive of all, the SASP has also been demonstrated to drive the initiation and progression of cancer through diverse effects. Several different cancer models have demonstrated the pleiotropic effects of the SASP, including the stimulation of angiogenesis (Coppe et al., 2006), the induction of cancer stem cells (Cahu et al., 2012), and the triggering of the epithelial-mesenchymal transition resulting in alterations to the tumor microenvironment, cellular dedifferentiation, and increased metastatic invasive capacity (Laberge et al., 2012). For example, MMP3 secreted by senescent fibroblasts promotes branching in mammary epithelial cells, and can potentially drive breast cancer metastasis (Parrinello et al., 2005). Further research shows that MMP proteins are also involved in tumor angiogenesis and epithelial-mesenchymal transition induction during the formation of carcinomas (Kessenbrock et al., 2010). Other SASP factors, growth-related oncogene and CXCR2, have been found to work together to induce esophageal cancer proliferation (Wang et al., 2006). Beyond cancer models, Yoshimoto et al. (2013) made a novel connection between obesity and the development of cancer. Surprisingly, they found that obesity leads to increased levels of deoxycholic acid in the gut, a known DNA damage-inducing agent. Deoxycholic acid then finds its way to the liver, where it induces the SASP and contributes to hepatocellular carcinoma progression in vivo (Yoshimoto et al. 2013). In perhaps the most direct demonstration of the pathogenic effects of senescent cells and the SASP in vivo, Baker et al. (2016) recently showed that genetically eliminating the naturally occurring senescent cells from normal mice resulted in delayed carcinogenesis and an extended lifespan, effects that may in part be due to decreased expression of the SASP in vivo.

Indeed, the ability of the SASP to promote carcinogenesis may be considered a potentially important mechanism for the striking age-related increases in the majority of human cancers (Childs et al., 2015; Krtolica et al., 2001). Thus, in certain acute contexts, SASP expression caused by DNA damage can lead to immune mediated clearance of oncogenic cells or work in an autocrine fashion to reinforce senescence. However, over time, chronic low-level inflammation driven by the SASP may work in a paracrine fashion to drive the initiation and progression of tumorigenesis (Figure 2). This paradox is hypothesized to be due to the effects of age-related "antagonistic pleiotropy" (Giaimo and d'Adda di Fagagna, 2012). This theory suggests that the SASP likely originally evolved for its developmental, wound healing and antitumor effects in younger tissues. However, the incessant accumulation of SASP-secreting senescent cells in older tissues, either due to decreased clearance or increased production, results in a chronic inflammatory state, ultimately altering the tissue microenvironment to favor tumorigenesis (Campisi, 2013; Childs et al., 2015).

The SASP and skin carcinogenesis

Fundamental discoveries in the senescence field have frequently been initially established in the skin. A classic study by Dimri et al. (1995) was the first to show that both skin fibroblasts and keratinocytes express greater levels of the senescence-associated marker β-galactosidase in aged human skin. These seminal observations lent credence to the role of senescent cells in normal physiology, and inspired further investigations into the biological mechanisms behind skin aging. Consistent with these early findings, later work demonstrated that the critical tumor suppressor and senescence mediator, p16, likewise accumulated in aged epidermis and dermis in vivo (Ressler et al., 2006). Further connecting senescence and skin aging, UVR is known to induce the DDR, SASP-like inflammation, and senescence in vitro (Shin et al., 2012), and is directly associated with human photoaging and p16 positivity in the skin in vivo (Nakanishi et al., 2009). Prominent SASP proteases such as MMP1 have been shown to degrade collagen in the skin and lead to UV-induced photoaging (Quan et al., 2013).

Aging is one of the greatest risk factors for the majority of human cancers (de Magalhaes, 2013), with skin cancer being no exception (Rogers et al., 2015). Chronic low-level inflammation that increases with aging, termed "inflammaging," is considered a possible driver of this dramatic age-related increase in malignant transformation (Franceschi and Campisi, 2014). Thus, the increased numbers of senescent cells and the resulting SASP in human tissues with aging have long provided a potential mechanistic link for this connection, as the SASP can lead to increased cancerous proliferation both in vitro and in vivo (Krtolica et al., 2001). Indeed, intrinsically aged, yet nonsenescent, human skin fibroblasts were recently shown to express numerous SASP genes, including IL1β, IL6, MMP1, MMP3, MMP10, *SERPINB2*, *CXCL10*, *AREG*, fibroblast growth factor-2, tumor necrosis factor-α, and vascular endothelial growth factor (Waldera Lupa et al., 2015). This underlying inflammatory microenvironment in aging and other settings of DNA damage may ultimately promote carcinogenesis in the skin (Figure 2). Wong et al. (2013) showed that exposure to chronic inflammation could drive invasive cutaneous carcinomas from diverse genetic and biological inciting events. Strikingly, SASP-like inflammation in the skin mediated by IL1β and transforming growth factor-β can drive the expression of

activation-induced cytidine deaminase, which leads to squamous cell carcinomas (SCCs) carrying Hras and Trp53 mutations, independent of any exposure to UVR (Nonaka et al., 2016). Further, a recent study demonstrated that activation of oncogenic Hras in the mouse epidermis has differential effects in young and old skin. Although young mice develop epidermal hyperplasia, old mice develop tumors consistent with SCC along with increased numbers of proinflammatory cytokines (Golomb et al., 2015). In a mouse model of cutaneous SCC, cancer-associated fibroblasts induce SASP-like inflammation, angiogenesis, and tumor growth through an NF-κB-mediated mechanism, reinforcing the links between the SASP and tumorigenesis in the skin (Erez et al., 2010). Consistent with these findings and a role for the SASP in skin carcinogenesis, inhibition of MMPs can prevent skin cancer invasion (Malaquin et al., 2013; Woenne et al., 2010). And finally, very recent work by Kaur et al. (2016) found that aged fibroblasts in the melanoma tumor microenvironment can secrete a Wnt antagonist, sFRP2, that can drive angiogenesis, metastasis, and resistance to targeted therapies such as vemurafenib.

Independent of aging, other forms of DNA damage such as UVR also promote senescence induction and inflammation (Figure 2) (Garibyan and Fisher, 2010). It has long been known that UVR-induced DNA damage can activate numerous canonical SASP genes ranging from IL1 and IL6 (Petit-Frere et al., 1998; Schwarz and Luger, 1989) to vascular endothelial growth factor and multiple MMPs (Dong et al., 2008; Ramos et al., 2004; Zhu et al., 2013). IL6 in particular has been shown to promote angiogenesis in basal cell carcinoma (Jee et al., 2004) and tumor invasion in SCC (Lederle et al., 2011). In addition, UVR has been shown to decrease Notch signaling and increase inflammation of the tissue microenvironment that drives field cancerization and SCC development (Hu et al., 2012). In melanoma, UVR-induced inflammation drives angiotropism and metastasis (Bald et al., 2014). Taken together, these findings suggest that inflammation due to the SASP and other DNA-damaging agents can have pathogenic roles in tumor progression in the skin.

The effects of the SASP do not stop at tumor promotion and progression, but may also lead to therapy resistance. The inhibitor of oncogenic $BRAF^{V600E}$ mutations, vemurafenib, can drive malignant melanocytes into stress-induced senescence rather than apoptosis (Haferkamp et al., 2013). Obenauf et al. (2015) showed that in melanoma, the therapeutic inhibition of BRAF and other similar oncogenes can stress drug-sensitive cells, leading to secretome changes, including the upregulation of NF-κB pathway genes, that create a permissive tumor microenvironment for the outgrowth of drug-resistance clones. These findings are consistent with the more recent demonstration that resistance to MAPK inhibitors is driven by transcriptional upregulation of NF-κB-driven inflammatory genes including *IL8* and *CCL8* (Hugo et al., 2015). Further, others showed that activated BRAF and NRAS in melanoma lead to tumor-promoting cyclooxygenase-dependent SASP-like inflammation (i.e., IL6, CXCL1) that also inhibits antitumor immunity (Zelenay et al., 2015). Together, these studies clearly implicate DNA damage/SASP-induced inflammation in not only the initiation and progression of skin carcinogenesis, but in metastasis and drug resistance as well. These studies provide rationale for further investigation exploring the role of SASP regulators ranging from p38MAPK and PKD1 to GATA4, mixed-lineage leukemia 1, and Brd4 in skin cancer development and prevention.

CONCLUSIONS

Our understanding of senescence and SASP has increased greatly over the previous decade, with a more detailed understanding of the mechanisms that regulate the expression of the SASP, its physiological effects, and how those effects can contribute to both skin cancer progression and therapy resistance (Childs et al., 2015). Despite this, the precise role of the SASP and SASP-like inflammation in the skin is still poorly delineated, though the cumulative evidence suggests that it plays a significant role in shaping the complex interactions between emerging tumors, the immune system, and the tissue microenvironment. Thus, it is imperative to continue to develop and refine our understanding of the double-edged sword that is the SASP, and how its inhibition or expression can lead to the initiation and progression of age-related pathologies, in particular cancer. As with many of the earliest and most seminal studies in the senescence field, the skin will undoubtedly continue to serve as a rich and very relevant model for this critical and complicated cellular process.

Abbreviations

ATM	ataxia telangiectasia mutated
DDR	DNA-damage response
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
mTOR	mammalian target of rapamycin
PKD1	protein kinase D1
SASP	senescence-associated secretory phenotype
SCC	squamous cell carcinoma

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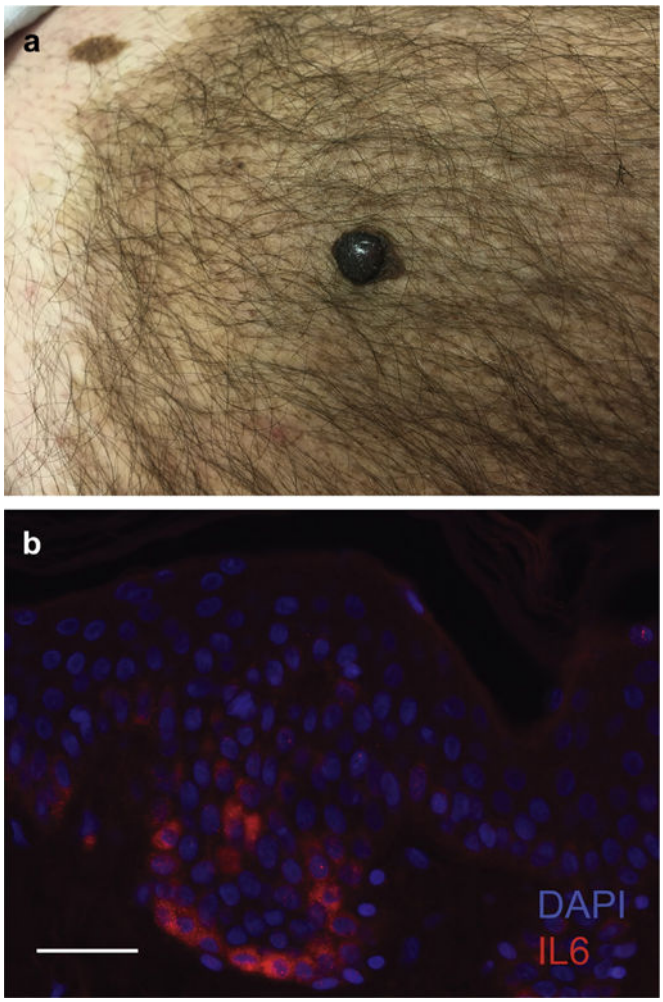

Figure 1. Senescence in the skin

(**a**) A malignant melanoma arising within a longstanding congenital nevus. Congenital nevi typically are driven into OIS by an activated *NRAS* oncogene. (**b**) A human melanocytic nevus displaying positive staining for the SASP cytokine, IL6, around the nevus melanocytes. OIS, oncogene-induced senescence; SASP, senescence-associated secretory phenotype.

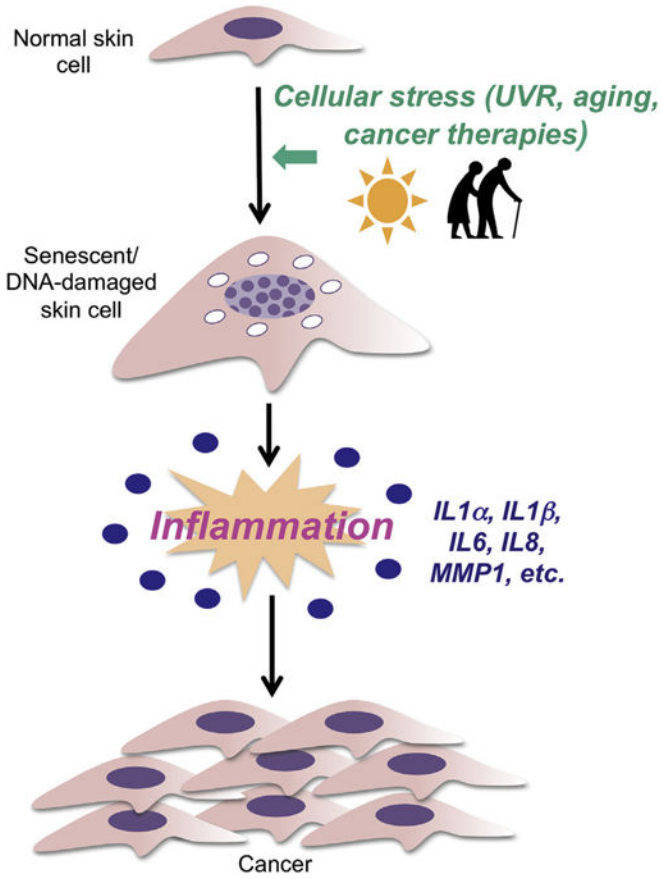

Figure 2. Senescent, damaged cell secretome can drive carcinogenesis

Diverse DNA-damaging stimuli, including UVR, telomere shortening with normal aging, activated oncogenes, and cancer therapies, can drive cells into senescence and lead to the secretion of the array of cytokines, chemokines, growth factors, and proteases known as the SASP. This can create a permissive tissue microenvironment that promotes the initiation, progression, and resistance of cancer cells. MMP1, matrix metalloproteinase 1; SASP, senescence-associated secretory phenotype.

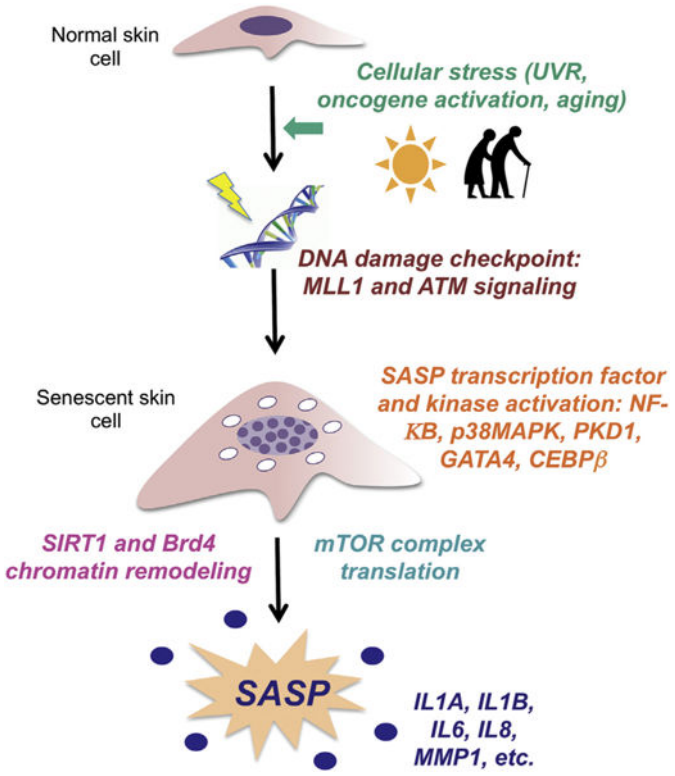

Figure 3. The SASP is regulated at multiple levels

When faced with senescence-inducing cellular stressors such as UVR, oncogene activation, and aging, the ATM-mediated DDR is activated during the S-phase checkpoint (for which MLL1 is required). The DDR in turn activates a number of downstream transcription factors and kinases, including NF-κB, p38MAPK, PKD1, CEBPβ, and GATA4. In conjunction with epigenetic remodeling (coordinated through chromatin regulators Sirt1 and Brd4), as well as translational regulation via mTOR, this results in massive upregulation of SASP cytokines, proteases, and growth factors. ATM, ataxia telangiectasia mutated; DDR, DNA-damage response; MAPK, mitogen-activated protein kinase; MLL1, mixed-lineage leukemia 1; MMP1, matrix metalloproteinase 1; mTOR, mammalian target of rapamycin; PKD1, protein kinase D1; SASP, senescence-associated secretory phenotype.