SASP: Tumor suppressor or promoter? Yes!

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Abstract

Cellular senescence is a permanent growth arrest in cells with damage or stress that could lead to transformation. Some tumor cells also undergo senescence in response to chemotherapy. Senescent cells secrete cytokines and other factors of the senescence-associated secretory phenotype (SASP) that contribute to tumor suppression by enforcing arrest and recruiting immune cells that remove these damaged or oncogene expressing cells from organisms. Some cells, however, can develop a SASP comprised of factors that are immunosuppressive and pro-tumorigenic by paracrine mechanisms. Likewise, the SASP in treated cancers can either contribute to durable responses or drive relapse. Here, we discuss the studies that have demonstrated a complex and often conflicting role for the SASP in tumorigenesis and treatment response.

Senescence suppresses tumorigenesis and contributes to chemotherapy response

Cellular senescence occurs when a normal cell is exposed to stress and enters a state of permanent growth arrest [1]. Unlike quiescence, cells that are senescent do not respond to mitotic cues to re-enter the cell cycle [2]. When in an arrested state, damaged or stressed cells cannot divide to form a tumor, thus, cellular senescence is tumor suppressive [3, 4]. Supporting this idea, many bona fide tumor suppressors that are mutated or inactivated in a variety of cancers are also directly involved in the senescence program, including *TP53*, *CDKN2A* (coding for p16) and *RB* [3, 5].

These tumor suppressor pathways are activated by stresses that might otherwise promote the transformation of a normal cell to a cancer cell. Some of these stresses include telomere shortening, DNA damage, and inappropriate oncogenic signals, such as those caused by mutant Ras [6]. While tumor suppression appears to be a primary function of senescence, accumulation of senescent cells may contribute to aging phenotypes [7]. In aged individuals, there is an increase in the presence of senescent cells and also age-related diseases [8]. Elimination of senescent cells in mouse models can ameliorate some aging phenotypes [9, 10].

Various “markers” are used to identify senescent cells in vitro and in vivo. These include effectors of senescence (activity of p53, expression of p21, p27, p16, p19, formation of gamma H2AX foci and heterochromatic foci) and phenotypes without a well understood function (senescence associated beta galactosidase [SA β Gal] staining, expression of DCR2, DEC1) [11, 12].

Similar to normal cells, many cancer cells retain the ability to undergo a senescent-like arrest following exposure to stresses such as DNA damage caused by chemotherapy or radiation treatments [13, 14].

While a cancer cell that is permanently arrested is hypothetically no longer a threat to relapse, in fact, tumors capable of undergoing senescence often relapse sooner than those that undergo cell death [5].

The undesirable properties of cellular senescence, specifically aging phenotypes and inferior response to chemotherapy treatments (discussed within), are likely at least partially attributable to the acquired ability of these cells to secrete a variety of cytokines, chemokines, growth factors, and proteases that comprise the Senescence-Associated Secretory Phenotype (SASP) [15].

Senescent cells acquire a secretory phenotype

In normal human diploid fibroblasts (HDFs), a repairable amount of DNA damage does not result in cytokine secretion [16]. More extensive DNA damage, however, causes a persistent DNA damage response (DDR) signal (for weeks) and initiation of the SASP [16]. Forced expression of the cell cycle inhibitor p16, however, which causes cells to adopt many characteristics of senescent cells without causing DNA damage, fails to induce the SASP [17].

Thus, the SASP program is activated by DNA damage and carried out by stress response kinases. Depletion of the kinases CHK2 or ATM, or the DDR component NBS1, abolishes expression of many SASP factors, including inflammatory cytokines IL6 and IL8, in HDFs undergoing oncogene induced senescence (OIS) [16, 18].

NFκB is a transcription factor complex responsible for transactivating many genes involved in inflammation [19]. In fact, NFκB is required for expression of numerous SASP factors during OIS of HDFs and other cells in culture [20-23]. In an unbiased screen, NFκB component RelA was found to associate with chromatin and promote transcription of SASP factors such as IL6, IL8 and CXCL1 in HDFs made senescent by oncogenic Ras [21]. In this model, knockdown of NFκB in combination with p53, but not NFκB alone, resulted in bypass of OIS [21]. Other fibroblast based studies have indicated a larger role for NFκB in senescence. In mouse and human fibroblasts, genetic loss of NFκB component RelA resulted in increased genomic instability and a higher rate of senescence bypass and immortalization [24]. In human mammary fibroblasts immortalized by hTERT and SV40 Large T antigen, subsequent inactivation of large T antigen (and thus activation of p53) induced senescence and NFκB was activated [22]. If NFκB components were depleted by siRNA, senescence was bypassed [25]. At present, it is not completely understood how NFκB is activated in senescent cells. p38MAPK is activated following stress in a manner temporally consistent with a role in the SASP, and phosphorylated p38MAPK activates NFκB and induces SASP independently of the DDR [26]. Further,

genes activated by ectopic p38MAPK have NFκB binding motifs in their promoters, and depletion of p38MAPK results in reduced expression of SASP factors such as IL6 in senescent cells [26]. Evidence also suggests ATM kinase and PARP are involved in NFκB activation [27], via regulation of the IKK complex [28, 29]. Other mediators of SASP gene expression include STAT transcription factors that are activated in many senescent cell types and regulate a subset of SASP genes [30-32], and GATA4, which promotes senescence when its degradation by autophagy is decreased during DNA damage [33].

In addition to being a tumor suppressive mechanism in normal cells, senescence is also a common outcome following chemotherapy treatment of *TP53* wild type cancer cells [13, 34, 35]. In cancer treatment, outcomes can be generally categorized as complete response (tumor eradicated), partial response/stable disease (tumor stops growing and/or shrinks some), or progressive disease/primary resistance (tumor never stops growing) [36]. Accumulating evidence from different cancers suggests that cellular senescence and the SASP are barriers to achieving a complete response [5, 37].

Many of the SASP genes are immune modulating cytokines and chemokines. These factors can interact with immune cells of either lymphoid or myeloid origin, and either contribute to tumor clearance or promote immunosuppressive environments. Lymphoid progenitors give rise to CD4+ helper and CD8+ cytotoxic T cells, which are involved in cell-mediated immunity [38]. Also of lymphoid origin, natural killer (NK) cells play a role in innate immunity and tumor surveillance, and natural killer T (NKT) cells have characteristics of both T and NK cells [38]. Myeloid progenitors give rise to, among other cell types, granulocytic myeloid- derived suppressor cells (MDSCs) and macrophages. MDSCs promote immunosuppressive environments and are associated with tumor progression [39]. Macrophages engulf cellular debris and can recruit lymphocytes. More specifically, M1 macrophages promote inflammation and are tumor suppressive, whereas M2 macrophages are anti-inflammatory and tumor promoting [38].

Because of the complex array of genes in the SASP, and variability of expression in different cell types, these secreted factors can either create an immunosuppressive, pro-tumor, chemoresistant environment or promote immune surveillance and tumor clearance.

The SASP as a tumor suppressor in normal or premalignant cells

The SASP was first described in studies to identify factors required for OIS in cultured HDFs. IL6 was found to be highly upregulated, and knockdown of IL6 expression using siRNA allowed normal cells to bypass OIS [40]. The secreted protein IGFBP7 and the chemokine receptor CXCR2 were identified in siRNA screens as required for OIS in HDFs [20, 41]. Inhibition of CXCR2 resulted in diminished

DNA damage response and bypass of senescence in HDFs after continuous passage in culture, after activation of the Ras pathway [20] or following radiation exposure [42]. Production of cytokines such as TGF- β and CCL2 can induce senescence in neighboring, non-senescent cells [43]. These studies show a tumor suppressive role for the SASP: promoting and maintaining the senescence program and permanently arresting and preventing transformation of normal cells with an oncogenic hit.

By necessity, the screens that identified the SASP were limited to relatively simple tissue culture model systems. Of course, SASP factors in organisms will likely act systemically and interact with other cell types and physiological systems, most notably, the immune system. If production of SASP cytokines can stimulate immune clearance of senescent cells, then this would result in tumor suppression similarly effective as apoptosis.

Indeed, Kang et al showed that when mice expressed activated Ras in the liver, the cells became senescent with SASP, and strong innate and adaptive immune reactions were stimulated. When Ras was induced in livers of mice lacking CD4⁺ T cells, progression to hepatocellular carcinoma (HCC) was accelerated [44]. Thus, immune clearance of senescent cells, likely via SASP, is critical for senescence mediated tumor suppression in this model. This was further tested by inhibition of the SASP regulator and chromatin modifier Brd4. Brd4 regulates the activation of NF κ B target SASP genes, and when inhibited pharmacologically or depleted genetically, SASP gene expression was diminished in senescent HDFs [45]. When mice with Ras induced in the liver as in Kang et al were treated with Brd4 inhibitors, immune cell infiltration and clearance of senescent cells was reduced [45]. Brd4 inhibitor effects on latency to carcinoma formation and survival were not tested. Subsequent studies have now shown that Ras-induced senescent liver cells secrete Ccl2 that recruits NK cells to assist in tumor clearance [46].

Immune response was also shown to promote tumor clearance in a mouse model system of p53 reactivation in HCC [47]. In this study, mice expressed inducible p53shRNA in the liver to drive HCC formation. When wild type p53 was re-expressed in the tumor cells, senescence and SASP factors were induced, and tumors regressed, but, curiously, apoptosis was minimal. When mice were treated with a macrophage toxin or antibodies to suppress neutrophils, both immune suppressing treatments inhibited tumor regression upon p53 reactivation, supporting the hypothesis that the innate immune system and the SASP contribute to tumor clearance [47]. Similarly, p53 wild type hepatic stellate cells made senescent by carbon tetrachloride treatment produced cytokines that elicit their immune clearance by NK cells [48] and they promote a tumor suppressive environment by recruiting M1 polarized

macrophages [49]. In mice with radiation exposure targeted to bone, senescence was induced and reinforced by the SASP factor IL6, NKT cells infiltrated and osteosarcoma formation was suppressed [50].

In a mouse model of Ras driven pancreatic cancer, premalignant lesions form with widespread senescence, SASP, and recruitment of tumor suppressive M1 macrophages [51]. When the NFκB component RelA was genetically ablated, the SASP in early tumors was abrogated, immune suppressive M2 macrophages and MDSCs infiltrated, tumorigenesis was accelerated, and survival was shortened. Downstream of NFκB, the Cxcl1-Cxcr2 pathway was shown to be required for the tumor suppressive role of the SASP in the same model [51]. Interestingly, the NFκB -Cxcl1-Cxcr2 axis switched from senescence promoting and tumor suppressive in premalignant lesions to tumor promoting in more advanced malignancies [51].

A “switch” was also observed in a model of colon tumorigenesis. Wnt dysregulation in the gut epithelium caused by loss of the casein kinase gene *Csnk1a1* results in widespread senescence and expression of a different set of inflammatory cytokines than a typical SASP, with no infiltration of immune cells [52]. These cytokines promoted growth arrest when p53 was wild type, but switched to proliferation and invasion promoting in p53 mutant tumor cells. NFκB pathway inhibition impaired senescence induction when p53 was wild type, but also blunted the tumor promoting effects when p53 was mutant [52].

While contributing to cell and non-cell autonomous tumor suppression, the SASP can also contribute to loss of function in organs. Marmary et al showed that mice that are exposed to high dose irradiation (IR) lost salivary gland function over 8 weeks. The radiated acinar cells did not undergo apoptosis, but instead became SAβGal positive and expressed senescence markers p21, Dcr2 and SASP factor IL6 [53]. In IL6^{-/-} mice, senescent phenotypes in the radiated salivary were rescued: Dcr2 and p19 were no longer expressed (though, p21 was), persistent H2AX foci were reduced and salivary function was partially restored [53]. Changes in tumors suppression as irradiated wild type and IL6^{-/-} mice aged were not examined.

The SASP as a tumor promoting factor in normal or premalignant cells

Different SASP factors have a variety of biological activities, including many that are pro-tumorigenic. In normal somatic cells, can the SASP drive the transformation of neighboring cells? Studies suggest a more likely scenario is that SASP factors drive proliferation, survival, and metastasis of already initialized or transformed cells. One of the earliest reports of a tumor promoting role for senescent cells

showed that soluble factors produced by senescent HDFs stimulated proliferation of different premalignant and fully transformed cell lines and accelerated tumorigenesis in xenografts [54]. Subsequent studies showed the promotion of tumorigenesis by SASP factors can be attributed to paracrine mitogenic or metastatic effects on other premalignant cells [55] and also interaction with surrounding endothelial cells, stromal cells and tissues, often mediated by matrix metalloproteinases (MMPs) [56].

Normal human epidermal keratinocytes undergo senescence in culture, with a low rate of spontaneous emergence and immortalization. When these keratinocytes were exposed to secreted factors from senescent dermal HDFs, they emerge at a higher frequency and exhibit epithelial to mesenchymal transition and MMP mediated migratory properties [57, 58]. Senescent normal human prostate epithelial cells can promote tumorigenesis in prostate cancer cells [59]. In a study of breast cancer progression, a small percentage of spontaneously occurring senescent cells in a population of HER2 expressing tumor cells could promote metastasis [60] and drive tumor growth in patient derived xenografts [61]. This effect was via production of IL6 that acted through the JAK/STAT pathway [61]. In a similar study, IL6 produced from senescent mesenchymal stem cells supported growth of breast tumors [62].

In the model systems used in these studies, it is evident that the presence of senescent cells and SASP factors are favorable for cell transformation, metastatic properties, and tumor growth. Importantly, however, many of these studies utilized immune compromised mice, and thus the positive or negative role of immune modulation by the SASP was not accounted for. In studies by Kang et al and Xue et al discussed above, the immune environment promoted tumor clearance. Recent studies have addressed this dearth.

A different and informative model of immune modulation by the SASP exists for prostate cancer, whereby tissue specific loss of Pten in mice causes premalignant lesions to develop with widespread senescence and SASP that, over time, can progress to invasive carcinoma [63, 64]. Using this model system, Toso et al showed that among the SASP factors produced by the senescent prostate cells were immune suppressive cytokines such as Cxcl2 and Gmcsf that are typically activated by Stat3. The benign prostate tumors were infiltrated with immunosuppressive MDSCs which inhibit proliferation of NK and CD8⁺ T cells [65] and produce IL1RA that antagonizes IL1 signaling and induction of senescence [66]. When Stat3 was genetically ablated, production of immunosuppressive cytokines was

reduced in the Pten^{-/-} prostate tumors, CD8⁺ T cells were recruited, and tumors were smaller and much less likely to develop into invasive carcinoma [65].

The findings in Toso et al were seemingly contradictory to the studies by Kang et al discussed above. However, this was clarified when the authors showed a different expression profile of SASP genes was induced after exposure to a different oncogenic stimulus. When prostate tumors were induced by Ras activation instead of Pten loss, the SASP and immune response resembled models of Ras-induced senescence in hepatocytes [44, 65]. These senescent cells had minimal activation of Stat3, reduced expression of immunosuppressive cytokines, and released cytokines such as IL1 and Ccl2 that attracted CD4⁺ T cells to the tumor microenvironment to promote tumor clearance [65]. This study demonstrated a tumor suppressive, immune surveillance role for the SASP factors that are typically regulated by NFκB, and a tumor promoting, immune suppressive role for the SASP factors regulated by Stat3. Another recent study showed Notch1 was induced during OIS of HDFs and in Ras induced liver cells, and negatively regulated pro-inflammatory cytokine expression, immune infiltration and senescence surveillance [67].

The finding that Stat3 mediates an immune suppressive environment was supported by a study using a mouse model of senescence induction by conditional p27 expression in stromal cells of mesenchymal origin [68]. The p27 induced cells in the skin expressed senescence and SASP genes, had phosphorylated Stat3, and infiltration of MDSCs accompanied by a decrease in lymphocytes. This immune suppressive environment promoted the growth of injected tumor cells in syngeneic, immune competent mice [68].

These studies suggest the possibility that inhibiting the activity of certain SASP factors could prevent incipient tumor cells from progressing, and several compounds have been shown to inhibit the SASP in various models systems. These include those that target p38MAPK [26], the NFκB pathway via IκB kinase (IKK) [20, 21], BRD4 [45] and MLL1, a chromatin modifier involved in DDR [69]. mTOR, which promotes SASP by facilitating translation of IL1A, an important initiating factor in the SASP [70], and by indirectly regulating degradation of SASP RNAs can also be targeted pharmacologically [71, 72]. Jak/Stat inhibition can be achieved by multiple small molecule inhibitors that have been shown to suppress the SASP and alleviate some aging phenotypes in mouse models [73]. Another strategy to abrogate the SASP is to eliminate senescent cells altogether by exploiting some vulnerability acquired during onset of the senescent phenotype. HDFs in OIS have increased expression of anti-apoptotic proteins such as BCL-W and BCL-2 [74], and drugs exist for the treatment

of chronic lymphocytic leukemia that target these proteins. ABT-263 and ABT-737 are mimetics of pro-apoptotic BCL2 family members and thus inhibit the anti-apoptotic BCL2 family members [75]. Senescent HDFs exhibited increased sensitivity to these drugs. Treatment of irradiated mice and normally aged mice with ABT-263 reduced senescent hematopoietic and muscle stem cells and alleviated premature aging phenotypes [76, 77]. Treating mice with ABT-737 eradicated senescent cells induced by DNA damage in the lungs, and in the epidermis caused by ectopic activation of p53 [74]. Elimination of senescent cells in the epidermis model rescued hair follicle stem cell proliferation [74]. Other compounds were recently discovered in an unbiased screen for toxicity to senescent cells. Dasatinib, a tyrosine kinase inhibitor, eliminated senescent fat progenitor cells in vivo and in vitro, and when combined with quercetin, a flavonol found in supplements, senescent cell burden was reduced in aged and radiation exposed progeroid mice [78]. These pharmacological SASP interventions have potential not just as “senolytics”, but as potential adjuvants in therapies that induce senescence in cancer cells (discussed below).

The SASP as a favorable factor in tumor treatment response

Tumors that are wild type for p53 often respond to DNA damaging chemotherapy (and other stresses) by entering senescence arrest instead of activating apoptosis. This arrest is characterized by the presence of many of the same features as senescence in normal cells: proliferative arrest with activation of p53 and expression of p21, SA β Gal staining and cell morphology changes. Since cells that are senescent are permanently arrested, it is thought to be beneficial to tumor response. It has now been thoroughly demonstrated that many types of tumor cells made senescent by chemotherapy treatment produce cytokines and chemokines of the SASP [18, 31, 32, 79-81]. What role the SASP plays in response is not clear: does it contribute to relapse by driving proliferation, survival and metastatic properties, or does it promote the durability of arrest and immune clearance of senescent tumor cells?

In one compelling study, inhibition of either aurora kinase A or CDK4/6 in mouse melanoma cells induced senescence and SASP and inhibited tumor growth in vivo [82]. The SASP factor Ccl5 mediated recruitment of CD4⁺ and CD8⁺ T-cells in the responsive tumors and response was best in immune competent mice. Enhancing T cell activation using anti CD137 agonist antibodies in combination with senescence induction achieved even more impressive outcomes, with no recurrence detected 12-months post therapy [82]. An NF κ B pathway inhibitor blocked senescence and the SASP induced in melanoma cells by aurora kinase inhibition and, at specific concentrations, diminished the response to

the aurora kinase inhibitor in xenografts, suggesting SASP factors were beneficial in this model [83]. In agreement with these studies, when lymphoma cells with an apoptotic block were treated with DNA damaging chemotherapy, if shRNA to NF κ B component RelA was induced, then activation of SASP genes was suppressed and fewer NK cells infiltrated the tumor. Mice with RelA knockdown were resistant to chemotherapy treatment and had reduced survival [21]. In these studies, the SASP driven by NF κ B improved response, maintaining arrest and attracting macrophages, NK and/or T cells to mediate immune clearance, thus leading to tumor regression.

SASP as a relapse promoting factor in tumor treatment

While senescence and a partial response/stable disease is certainly preferable to primary resistance, growing evidence suggests wild type p53 mediated arrest and induction of cellular senescence is a barrier to complete eradication of the tumor with chemotherapy and can contribute to relapse. This is supported by clinical studies from cancers of the breast [34, 84-88], ovary [89, 90], lung [91], mesothelium [92], colon [93], stomach [94], bladder [95], rectum [96, 97] and in mouse models [31, 98].

Many SASP factors have been shown under various conditions to induce tumorigenic properties such as proliferation, survival, angiogenesis, and metastatic potential [99-104]. The SASP can also cause the emergence of cancer stem-like cells which increase tumorigenicity and drug resistance [32, 81, 105, 106].

SASP cytokines CXCL1 and 2 are also induced in breast cancer cells by chemotherapy and mediate chemoresistance by triggering a paracrine cascade that stromal cells respond to. Combining chemotherapy with an inhibitor of CXCR2 (receptor for CXCL1/2) reduced metastatic burden in a xenograft model, suggesting that the SASP cytokine network contributes to the failure of chemotherapy [79].

Microphthalmia-associated transcription factor (MITF) is expressed in melanocyte lineage and is required for proliferation of some melanomas. Conditioned medium from melanoma cells made senescent by knockdown of MITF stimulates xenograft tumor growth, likely via NF κ B dependent expression of CCL2 [23]. Similarly, when glioblastoma (GBM) cells exposed to IR become senescent with SASP, these cells promote tumor growth of non-senescent GBM cells in xenografts [107].

Further, Stat3 activity mediated by SASP cytokines can promote survival and immuno-suppressive environments that prevent tumor regression. Toso et al showed that the combination of different

cytokines expressed in senescence dictate whether response to chemotherapy is helped or hindered by the SASP. Senescence was enhanced in Pten^{-/-} prostate tumors by treatment with the chemotherapy drug taxol [65]. As discussed earlier, benign Pten^{-/-} prostate tumors have an immune suppressive environment induced by SASP activated Stat3. When the Jak/Stat axis was blocked pharmacologically in the tumor bearing mice, the SASP profile was altered, an active T cell immune response was initiated, and response to taxol was markedly improved [65]. STAT3 signaling in malignant pleural mesothelioma can enrich for cell populations that are chemoresistant and knockdown of STAT3 inhibits expression of epithelial-to-mesenchymal genes normally induced by SASP [32]. Endothelial cells in the thymus made senescent with SASP by chemotherapy can protect lymphoma cells by a p38MAPK/IL6/Jak/Stat pathway and contribute to relapse [108]. These studies, taken together, demonstrate the complexity of the SASP network of factors and show that tumor cells made senescent by chemotherapy can ultimately have a pro-tumorigenic effect in cancer (Figure 1, Key Figure). However, pharmacological modulation of the SASP (by Jak inhibition) can promote immune activity and enhance the response to chemotherapy. Thus, numerous in vivo studies show the SASP has contradictory roles in drug response and tumorigenesis (Table 1).

Concluding remarks

In sum, the SASP is a complex network of secreted factors that can have a net positive or negative effect on cancer development and drug response (Figure 1, Key Figure). Evidence suggests that when a strong immune response is initiated, outcomes are better and the good outweighs the bad. If, however, cytokines are immune suppressive, as was shown in Toso et al through the Stat3 pathway, then the net effect is negative and response will be worse (Table 1). Interestingly, in Jackson et al, where chemotherapy treated senescent tumors that acquired the SASP responded poorly and relapsed quickly, Stat3 was strongly activated, and immunosuppressive cytokines were expressed [31]. It is reasonable to speculate that in tumor types where p53 is wild type and complete responses are rare [34, 84-97], an SASP mediated immune response is weak or tumor promoting, and pharmacological intervention is warranted (see Outstanding Questions). In the final analysis, it seems targeting the SASP will be much like any other targeted therapy: effective in a subset of tumors, and identification of that subset will be critical.

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Figure 1, Key Figure. Model for the conflicting roles of senescence and the SASP in tumor suppression and drug response.

Exposure of normal cells and some cancer cells to stresses such as DNA damage activates pathways leading to p53 mediated arrest and NFκB mediated secretion of cytokines and chemokines.

A. NF-κB mediates the tumor suppressive role of SASP in normal or premalignant cells by transactivating cytokines that stimulate immune cells that clear the damaged cells and reinforce growth arrest.

B. In some normal or premalignant cells, the SASP can be tumor promoting by activating the JAK/STAT pathway which induces immunosuppressive cytokines. SASP factors can also be mitogenic and pro-survival, and promote angiogenesis and reorganization of stroma.

C. In p53 wild type cancer cells treated with DNA damaging chemotherapy, the SASP can activate the JAK/STAT and other pathways to promote cell survival, proliferation, increase in cell stemness and an immunosuppressive environment, thus causing relapse.

D. In contrast, in some model systems, the SASP can attract immune cells through the NFκB pathway to facilitate in tumor clearance, and this can be enhanced via T cell therapies.

The presence of the same cytokines in areas depicting tumor growth and tumor suppression demonstrates the complexity and tissue and stimulus specificity of the SASP.

<u>Senescence stimulus</u>	<u>SASP cell/tissue</u>	<u>Target of SASP</u>	<u>Outcome</u>	<u>Experimental Modification</u>	<u>Experimental Outcome</u>	<u>Notes</u>	<u>Reference</u>
Ras	hepatocyte	CD4+T cells	senescent cell clearance/tumor suppressed	cross to CD4-/-	tumor promotion	senescent hepatocytes accumulate under immunosuppressive therapy	[44]
p53 reactivation	Hepatocellular carcinoma	Macrophage	senescent cell clearance/tumor regression	immune suppressing Rx	tumor persistence	p53 reactivation upregulated neutrophils and NK cells near HCC	[47]
Carbon tetrachloride	hepatic stellate cells	NK cells, M1 macrophages	senescent cell clearance/fibrosis reduced	cross to p53-/-; Cdkn2a-/-	Increased liver fibrosis	Senescent hepatic stellate cells are targeted by NK cells	[48]
radiation	osteoblasts	NKT cells	tumor suppression	Cross to IL6-/-	tumor promotion shorter survival	RB1 also required for senescence and immune cell infiltration	[50]
Ras	pancreas	M1 macrophages	tumor suppression	Cross to RelA-/-	Immune suppression, tumor promotion	Cxcl1/2 required for suppression	[51]
Ras	prostate	CD4+T cells	tumor suppression	N/A	N/A	IL1 and Ccl2 implicated in immune response	[65]
Wnt activation	colon	colon	durable growth arrest	IKK inhibitor	impaired senescence induction	p53null tumors stimulated by SASP	[52]
radiation	salivary gland	salivary gland	loss of salivary function	Cross to IL6-/-	partial rescue of salivary function		[53]
chemotherapy	thymic endothelial	lymphoma	Lymphoma relapse and short survival	Jak/Stat inhibiting Rx	Improved response, extended survival	IL-6 mediates induction of BclXL promoting cell survival	[102]
Pten loss	prostate	MDSCs	tumor promotion	Cross to Stat3-/-	CD8+ T cell recruited, tumor suppression	GMCSF and Cxcl1 implicated in immune suppression	[65]
Docetaxol	prostate tumor	MDSCs	tumor promotion	Jak/Stat inhibiting Rx	CD8+ T cell recruited, response improved	Senescent cells cleared in mice treated with JAK2 inhibitor plus taxol	[65]

Table 1: The SASP promotes or suppresses tumorigenesis and drug response in immune competent model systems

A. Tumor suppressive factor in normal or premalignant cells

C. Relapse promoting factor in cancer treatment

Telomere attrition
Oncogenic signaling

Senescence

Senescence

Chemotherapy

B. Tumor promoting factor in normal or premalignant cells

D. Favorable factor in cancer treatment