Cellular Senescence and the Immune System in Cancer

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Abstract

In response to a variety of cancer-inducing stresses, cells may engage a stable cell cycle arrest mechanism, termed cellular senescence, to suppress the proliferation of preneoplastic cells. Despite this cell intrinsic tumor suppression, senescent cells have also been implicated as active contributors to tumorigenesis by extrinsically promoting many hallmarks of cancer, including evasion of the immune system. Here, we discuss these dual, and seemingly contradictory, roles of senescence during tumorigenesis. Furthermore, we highlight findings of how senescent cells can influence the immune system and discuss the possibility that immune cells themselves may be acquiring senescence-associated alterations. Lastly, we discuss how senescent cell avoidance or clearance may impact pathology.

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Introduction

Most spontaneous human malignancies display an increased risk and incidence with advancing age. The process of tumorigenesis typically requires multiple steps

that progressively evolve to create a neoplastic state. In recent years, new hallmarks and characteristics of cancer, including avoidance of immune detection and tumor-promoting inflammation, have become appreciated. These hallmarks highlight the importance of the immune system's influence on tumor cell survival, proliferation, and dissemination. While there has been extensive investigation regarding the various stages of tumorigenesis, there are multiple areas in which cancer is still poorly understood.

During an organisms' lifetime, their cells are exposed to a variety of stresses, both internal and external. Under certain circumstances when damage is unrepairable, cells may go through the process of programmed cell death termed apoptosis. Alternatively, these cells may stably exit the cell cycle and become senescent. There are several distinguishing characteristics of senescent cells (Fig. 1), including elevated expression of the cell cycle inhibitor p16^{Ink4a} (hereafter p16), β -galactosidase activity at pH 6.0 (SA- β -Gal), and a distinctive secretory phenotype that involves chemokines, cytokines, metalloproteinases, and growth factors that are collectively known as the senescence-associated secretory phenotype (SASP) [1]. It is important to note while these features typically associated with senescence, they can be observed in a variety of other biological contexts.

Recent studies have established that senescent cells actively contribute to aging [1]. Additionally, senescent

Fig. 1. Senescence as both an anti-tumorigenic and a protumorigenic mechanism. Senescent cells have the ability to play a dual role in promoting and attenuating cancer. In response to a variety of stresses, they enter a durable cell cycle arrest to suppress proliferation. Senescent cells can display a variety of features, including elevated reactive oxygen species, cell cycle regulators (such as p16 and p21), senescence-associated heterochromatin, and the SASP, which includes immunostimulatory cytokines, chemokines to recruit lymphocytes, and metalloproteinases (7, 9, and 12) that coun-

ter tumor formation. However, the SASP can also perturb the surrounding environment through the secretion of these same factors that can be immunosuppressive. ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; TGF, transforming growth factor; MMP, matrix metalloproteinases; MCP, membrane cofactor protein; TNF, tumor necrosis factor; CCL, chemokine ligand; SAHF, senescence-associated heterochromatin; IL, interleukin.

cells have been observed in neoplastic tissues [2]. In these contexts, senescence can be driven through either excessive oncogenic signaling or loss of certain tumor suppressor genes [3]. Using both murine and human fibroblasts, excessive *Ras* activity is capable of promoting oncogene-induced senescence (OIS), primarily through Rb- and p53-dependent pathways [3]. This durable cell cycle arrest of senescent cells would largely function as a tumor preventive mechanism. OIS also engages a DNA damage response while activating tumor suppressor pathways, including p53-mediated transcription of the cell cycle inhibitory protein p21^{Cip1} (hereafter p21). Additionally, OIS has also been observed in several human pathologies including early-stage prostate tumors, colon adenomas, astrocytoma, neurofibromas, and benign neoplasms of melanocytes. Although OIS is supported by multiple murine and human studies, the interplay between tumor cells, senescent cells, and the immune sys-

tem is only beginning to be elucidated. Additionally, the significance of senescent cells to potentially contribute to the process of tumorigenesis through extrinsic signaling remains poorly understood. In this review, we will discuss the role of senescence not only as an anticancer effect but also as a potential promoter of tumorigenesis. Moreover, we will focus on the relationship between the immune system and senescent cells and how senescent cells could become a new therapeutic target to combat cancer.

Senescent Cells: Against, Yet in Favor of Malignancy?

Historically and from a biological perspective, due to their persistent cell cycle arrest, senescence is commonly thought to function as a potent tumor-suppressive mechanism; however, it may also promote tumor formation

(Fig. 1). Using *in vivo* models of hepatocellular carcinoma, senescence induction of liver tumor cells through the restoration of p53 promotes clearance of these cells by the innate immune system [4]. Conversely, the absence of p53 bypasses senescence, thereby permitting the transformation of hepatocytes into cancer cells. These results demonstrate that senescence arrest, both before and after tumor establishment, can be beneficial to protect against malignancies. Consistent with this idea, several drugs have been generated to promote senescence in neoplastic cells. For example, CDK4/6 inhibitors are currently being used in preclinical and clinical trials to treat solid and liquid tumors [5].

While cellular senescence intrinsically suppresses tumorigenesis of preneoplastic cells, the SASP produced by senescent cells can in fact extrinsically promote tumor growth, relapse, and metastasis [6–8]. Recent studies have demonstrated that the SASP can be controlled in a temporal manner. Early SASP factors, including transforming growth factor (TGF)- β 1 and TGF- β 3, are immunosuppressive, whereas the subsequent SASP consists of proinflammatory cytokines, including interleukin (IL)-6, IL-1 β , and IL-8 [9, 10]. Senescent stromal cells may influence tumorigenesis through the secretion of IL-6, which recruits myeloid suppressive cells to inhibit T-cells response against malignant cells [7]. Differences in NAD⁺ metabolism can influence p38 MAPK and NF- κ B (nuclear factor kappa B) signaling, which in turn impacts proinflammatory SASP production [11]. Additionally, senescent cells also potentiate their detrimental effects by activation of the cGAS-STING pathway, which triggers the production of SASP factors that promote paracrine senescence [12]. In response to chemotherapy, senescent cells accumulate and promote the adverse effects typically associated with therapeutic interventions [6]. Overall, these results demonstrate that senescent cells can detrimentally influence tissue homeostasis in ways that can potentiate cancer.

The Immune System, Senescence, and Cancer

The immune system is the body's most powerful line of defense to battle foreign agents and neoplastic cells. Some successful immunotherapeutic regimens are based on checkpoint inhibitors that can help a subset of cancer patients to have a durable tumor response. Chimeric antigen receptor-T-cell therapy utilizes peripheral T-cells that are engineered to express synthetic T-cell receptors to selectively target tumor-surface antigens. Chimeric

antigen receptor-T cells have shown some benefit for treating hematological cancers, but the successful application for solid tumors has been less promising [13]. Recently, a new approach emerged using models of breast carcinoma to demonstrate that CDK4/6 inhibition not only induces tumor cell cycle arrest as expected but also promotes cytotoxic T-cell-mediated clearance of tumor cells by activating endogenous retroviral elements [14]. Interestingly, CDK4/6 inhibition did not repress the expansion of T-cells; instead, it augmented the effect of PD-1 inhibition. PD-L1 stability is regulated through the cullin 3-SPOP E3 ligase via proteasome-mediated degradation by cyclin D-CDK4 [15]. T-regulatory cells are more susceptible to CDK4/6 inhibition due to their higher expression of CDK6 than other T-cell subtypes, which in turn leads to a higher expression of cytotoxic CD8⁺ T cells to respond to cancer cells [16]. Collectively, these findings suggest that combinatorial therapies using CDK4/6 inhibitors and PD-L1 immune blockade may enhance the efficacy of immunotherapies in cancer patients.

Although CDK4/6 inhibition can be considered a fail-safe program, it is important to recognize that senescence has also been implicated in B-cell malignancies. B-cells isolated from old bone marrow express higher amounts of p16 and p19, which suggests that cell cycle arrest and senescence may occur *in vivo* [17], which may reduce the possibility for leukemia formation with advancing age. In contrast, studies from E μ -Myc transgenic mice have demonstrated a “stemness” signature in senescent B-cell lymphomas expressing upregulated Wnt signaling [18]. Moreover, after chemotherapy treatment in switchable models of senescence targeting H3K9me3 or p53 to allow the escape of cellular arrest, it was found that previously chemotherapy-induced senescent cells exit cell cycle arrest and proliferate with an enhanced Wnt-signaling having a higher tumor initiation potential. Surprisingly, the enforcement of senescence in models of acute lymphoblastic leukemia and acute myeloid leukemia reprogram non-stem leukemia cells into self-renewing stem cells [18]. These data propose an unexpected link between senescence and cancer stemness. Therefore, it will be interesting to determine if nonneoplastic cells are similarly equipped with “stemness” capacity and how senescence triggers this capacity.

The impact of senescence in the immune system is only beginning to be elucidated. Since there is currently no universal marker for senescence, the contribution of senescence *in vivo* has been explored using a luciferase knock-in mouse model in the endogenous p16 gene

(p16LUC) [19]. Interestingly, p16⁺/SA- β -Gal⁺ macrophages were found to accumulate in chronological aged p16LUC mice. Additionally, implantation of alginate bead-embedded senescent cells into the peritoneal cavity of p16LUC mice potentially recruits macrophages with features resembling senescence through the SASP, which have been called senescence-associated macrophages (SAMS) [19]. However, it was later reported that adipose tissue macrophages express p16 and SA- β -Gal as part of a normal physiological response to immune stimuli and are not indicative of senescence per se [20]. To further confuse the issue, use of a new p16 reporter allele, p16^{tdTom}, in combination with an injection of neonatal dermal fibroblast-loaded alginate beads promoted the acquisition of p16 and SA- β -Gal in macrophages and resulted in functional and transcriptional features indicative of senescence [21].

In the context of disease, macrophages expressing SA- β -Gal and p16 may accumulate and contribute to senescence-associated pathology. For example, during atherosclerosis, circulating monocytes accumulate and differentiate into foam macrophages, which then promote atherosclerosis. Subsequently, use of transgenic and pharmacological approaches for the removal of foamy p16⁺ senescent macrophages blunted plaque accumulation and maladaptive plaque remodeling processes, which helped alleviate the pathology [22]. Alveolar macrophages cause an alveolar collapse in an elastase-induced emphysema model [23]. Removal of p19^{ARF}-expressing senescent cells protects against pulmonary emphysema in this model [24].

In cancer, immunosuppressive macrophages undergo molecular adaptations that suppress the infiltration of T-cells into the tumor microenvironment. This results in an impaired response against tumors cells, while also promoting neoplastic progression through the secretion of an array of proinflammatory factors [25]. There are mainly 2 types of macrophages: M1 and M2. M1 macrophages are involved in responding against pathogens or injury and stimulate the immune response. On the other hand, M2 macrophages are part of wound healing, immune suppression, and angiogenesis. For this reason, most tumor-associated macrophages are collectively thought of to be M2. Establishing strategies that not only inhibit macrophage-mediated immunosuppression but also prevent growth stimulation could therefore prove useful. Innovative strategies have targeted PI3K γ in macrophages to show that its inactivation stimulates and prolongs NF- κ B activation and inhibits C/EBP β , thereby leading to polarization of mac-

rophages and secretion of factors that recruit CD8⁺ T-cells to target cancer cells [26]. Currently, this theory has been found to synergize with anti-PD1 immunotherapy and is the basis of phase I clinical trial in patients with advanced solid tumors.

Additional studies have suggested that resident and bone-derived macrophages can accumulate in tumors. It is not clear whether resident or infiltrating macrophages have a similar impact in promoting their adverse effects. In models of pancreatic ductal adenocarcinoma, subsets of resident macrophages are proliferative and immunosuppressive, which is thought to promote tumor progression. Furthermore, these cells exhibit a profibrotic transcriptional profile, which potentially reflects their capacity of producing and remodeling the extracellular matrix [27].

An emerging topic in the field is immunometabolism, although this topic is understudied, the relevance to macrophages playing a dual role in cancer is very apparent. It is known that the PI3K/Akt/mTOR pathway promotes L-arginine, which is an important feature of altered metabolism that leads to the immunosuppression aspect of tumor-associated macrophages [28]. Interestingly, in senescent cells, mTOR inhibition suppressed IL1A diminishing NF- κ B and thereby suppressing the establishment and maintenance of the SASP [29]. Moreover, in K-ras^{LA1} mice, which are predisposed to early-onset lung cancer, it was reported that mTOR inhibition reduced the size and number of lung adenomas and blocked malignant progression by inducing apoptosis of intraepithelial macrophages [30]. Collectively, these findings suggest that mTOR-dependent signaling plays an important role in controlling the fate of tumor-associated macrophages.

Clearly, the role of macrophages in geriatrics and age-related pathologies is beginning to be elucidated, but the establishment, if they are becoming fully senescent or simply having a phenotype reminiscent of senescence, remains unclear. It is important to establish if SAMS contribute actively to tumorigenesis and determine how SAMS influence different cell types within the tumor microenvironment (Fig. 2). We are only beginning to understand the importance of the immune system and its relationship to senescence and cancer. However, it is imperative to distinguish the interplay between the innate and the adaptive immune response against or in favor of malignancies and determine if senescence is changing the actions of the immune system from an anticancer to a pro-tumorigenesis response.

Fig. 2. Tumor-associated macrophage display features of senescence. Oncogene-induced senescent cells, through the secretion of SASP factors, may promote senescence in cells of the immune system, including macrophages, in an autocrine fashion. Subsequently, senescent-associated macrophages may influence other cells of the immune system to evade tumor cell surveillance and senescent

cell clearance. Additionally, the accumulation of senescent cells will promote the release of SASP factors that foster tumor cell growth. SASP, senescence-associated secretory phenotype; TGF, transforming growth factor; TNF, tumor necrosis factor; CCL, chemokine ligand; PDGF, platelet derived growth factor; IL, interleukin.

In sum, the mechanisms by which the immune system confronts tumor cells are now better understood and have opened the door for new therapies. A novel strategic approach is to employ our current understanding of cellular senescence and the immune response to apply combination therapies. First, inducing senescence in transformed cells and then harnessing the immune system to eliminate these cells may hold promise for future therapies.

Avoidance of the Senescence Program

Avoiding or preventing the senescence program through the inactivation of tumor suppressors in mice, namely p16, leads to the premature death of mice from cancer [31]. p16 has also been linked to being a marker of tumor subtypes, and high p16 levels are a marker of early-stage small-cell lung cancer [32]. Perhaps, this induction is indicative of senescence induction as a cancer defense mechanism in this context. Alternatively, p16 and p21, genes implicated in the cell cycle arrest underlying senescence, promote tumor growth through ex-

trinsic effects by upregulating CX3CR1 expression and conferring resistance to antitumor immune responses [33]. Monocyte myeloid-derived suppressor cells express high levels of p16 and p21, which stimulate CX3CR1 chemokine receptor expression by preventing CDK phosphorylation and inactivation of SMAD3. Ablation of p16 and p21 reduces CX3CR1 expression and prevents the accumulation of monocyte myeloid-derived suppressor cells, thereby driving tumor progression. These results suggest the implication of senescence as a driver mechanism of tumorigenesis. In fact, p16 expression has been detected in the tumor stroma and is associated with high risk of recurrence or poor survival in different types of cancer. In regard to the role of p16 in myeloid cells, it is known that p16 induce macrophages toward an inflammatory state, which correlates with chronic inflammation under the hallmarks of cancer. Support of this idea comes from studies where clearance of naturally occurring p16⁺ senescent cells delayed tumor formation [1], indicating that senescent cells can be detrimental for tissue homeostasis and promote tumor formation.

Strategic and Tactical Therapeutic Actions to Clear Senescent Cells

Senescent cells can act as tumor suppressors, yet potentiate tumor progression through the secretion of a variety of different SASP factors. Therefore, it is important to develop strategies to either selectively remove senescent cells after they have engaged their antineoplastic response or attenuate their extrinsic signaling through modulation of the SASP. Recent studies have used transgenic mice to inducibly eliminate p16-expressing senescent cells in contexts of aging and age-related diseases including atherosclerosis [22], osteoarthritis [34], and neurodegeneration [35]. Therapy-induced senescence was studied by using a transgenic approach to eliminate and determine the impact of p16 cells (p16-3MR) in the context of tumor metastasis and relapse. Remarkably, the elimination of senescent cells ameliorates the adverse effects of doxorubicin, a chemotherapeutic agent, and reduced tumor metastasis and recurrence [6]. It is important to note that although the dosages of chemotherapy administered to research rodents and humans are not the same, the pharmacodynamics, as opposed as pharmacokinetics, can be similar. Therefore, it is reasonable to suggest that chemotherapy may induce senescent cell accumulation in patients and contribute to side effects associated with chemotherapies. Importantly, these studies consistently demonstrate that removal of senescent cells has a potentiating effect on disease and pathology.

With these results in mind, efforts have been made to selectively remove senescent cells in the absence of genetic modifications through pharmacological approaches referred to as senolytics. Senotherapy encompasses 3 main categories: permanent removal of senescent cells (senolysis), immune-mediated senescent cell clearance, and SASP neutralization [36]. ABT-263 (navitoclax), a BCL (B cell lymphoma)-2-specific BH3 mimetic drug, was shown to deplete senescent hematopoietic stem cells, senescent muscle stem cells [37], and senescent glial cells [35]. Cancer cells, paradoxically, are primed to die. Stress and mutations upregulate their levels of BH3-only protein, imposing pressure to elevate the levels of BCL-2 antiapoptotic proteins [38]. As a result, ABT-263 is a potent drug to induce apoptosis in cancer cells. Therefore, in the context of solid tumors, it is important to distinguish the efficacy of ABT-263 against cancer and senescent cells.

Additional targets have been found that may be able to be exploited to demonstrate senolytic properties. Forkhead box protein O4 interacts strongly with p53 in senescent cells, which is thought to prevent p53-mediated apoptosis. Administration of a peptide that disrupted this interaction

in mouse models of aging restored fitness, fur density, and kidney function [39]. Furthermore, 2 inhibitors of the heat shock protein 90 family were identified to have senolytic properties in mouse and human cells [40]. However, it is important to note that although heat shock protein 90 can eliminate senescent cells, it can also cause a major impact in normal processes, such as assisting in protein folding and stabilizing proteins against heat stress. Therefore, more research should be done to determine to what extent these drugs benefit rather than affect normal physiology.

On the other hand, therapy-induced senescence can represent a novel approach to fight against cancer. It has been proposed as a 2-step target: first, induce immortal cells into the senescence program and then clear the senescent cells with some senolytic interventions [41]. However, this approach should be taken with precaution since senescent cells have both beneficial and detrimental roles in cancer. In addition, it will be important to design senotherapies that can modulate particular SASP factors that are detrimental for the tissue homeostasis, while allowing the tumor suppressor mechanism to remain intact in senescent cells. Overall, additional research using preclinical animal models is necessary to study and determine the efficacy of these treatments.

Conclusion and Future Directions

As the alchemist, Paracelsus stated, “the dose makes the poison.” High levels of a certain oncogene, such as Ras, can promote senescence instead of driving these cells to become precursors of vigorous tumors. Initially, senescent cells, because of their durable cell cycle arrest, can act as tumor suppressors to halt the proliferation of cells. However, cells that undergo senescence by oncogene expression release a variety of SASP factors including several ILs, IGFBPs (insulin-like growth factor binding protein), and TGF- β . Subsequently, SASP factors can act in a paracrine fashion to induce senescence in adjacent cells and in an autocrine fashion to recruit proinflammatory cells that can promote tumor growth. Ongoing efforts are being made to decipher the interplay between cancer cells, senescent cells, and immune cells. In addition, it is important to establish what is the involvement of senescence in some immune cells including macrophages. To this end, new tools are required to investigate this phenomenon and induce senescence to study their cellular behavior under different contexts such as tumorigenesis. In the future, senolytics that selectively eliminate senescence cells in a localized manner may hold novel interventional opportunities in cancer.

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Disclosure Statement

D.J.B. is a co-inventor on patent applications licensed to or filed by Unity Biotechnology, a company developing senolytics medicines, including small molecules that selectively eliminate senescent cells. Research in the Baker laboratory has been reviewed by the Mayo Clinic Conflict of Interest Review Board and is being conducted in compliance with Mayo Clinic Conflict of Interest policies.

References

- Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature*. 2016 Feb;530(7589):184–9.
- Collado M, Gil J, Efeyan A, Guerra C, Schumacher AJ, Barradas M, et al. Tumour biology: senescence in premalignant tumours. *Nature*. 2005 Aug;436(7051):642.
- Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*. 1997 Mar;88(5):593–602.
- Xue W, Zender L, Miething C, Dickins RA, Hernandez E, Krizhanovsky V, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature*. 2007 Feb;445(7128):656–60.
- Georger B, Bourdeaut F, DuBois SG, Fischer M, Geller JL, Gottardo NG, et al. A Phase I Study of the CDK4/6 Inhibitor Ribociclib (LEE011) in Pediatric Patients with Malignant Rhabdoid Tumors, Neuroblastoma, and Other Solid Tumors. *Clin Cancer Res*. 2017 May;23(10):2433–41.
- Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. *Cancer Discov*. 2017 Feb;7(2):165–76.
- Ruhland MK, Loza AJ, Capietto AH, Luo X, Knolhoff BL, Flanagan KC, et al. Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. *Nat Commun*. 2016 Jun;7(1):11762.
- Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci USA*. 2001 Oct;98(21):12072–7.
- Hoare M, Ito Y, Kang TW, Weekes MP, Matheson NJ, Patten DA, et al. NOTCH1 mediates a switch between two distinct secretomes during senescence. *Nat Cell Biol*. 2016 Sep;18(9):979–92.
- Ito Y, Hoare M, Narita M. Spatial and Temporal Control of Senescence. *Trends Cell Biol*. 2017 Nov;27(11):820–32.
- Nacarelli T, Lau L, Fukumoto T, Zundell J, Fatkhutdinov N, Wu S, et al. NAD⁺ metabolism governs the proinflammatory senescence-associated secretome. *Nat Cell Biol*. 2019 Mar;21(3):397–407.
- Glück S, Guey B, Gulen MF, Wolter K, Kang TW, Schmacke NA, et al. Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. *Nat Cell Biol*. 2017 Sep;19(9):1061–70.
- Akce M, Zaidi MY, Waller EK, El-Rayes BF, Lesinski GB. The Potential of CAR T Cell Therapy in Pancreatic Cancer. *Front Immunol*. 2018 Sep;9:2166.
- Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, et al. CDK4/6 inhibition triggers anti-tumour immunity. *Nature*. 2017 Aug;548(7668):471–5.
- Zhang J, Bu X, Wang H, Zhu Y, Geng Y, Nihira NT, et al. Cyclin D-CDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance. *Nature*. 2018 Jan;553(7686):91–5.
- Deng J, Wang ES, Jenkins RW, Li S, Dries R, Yates K, et al. CDK4/6 Inhibition Augments Antitumor Immunity by Enhancing T-cell Activation. *Cancer Discov*. 2018 Feb;8(2):216–33.
- Signer RA, Montecino-Rodriguez E, Witte ON, Dorshkind K. Aging and cancer resistance in lymphoid progenitors are linked processes conferred by p16INK4a and Arf. *Genes Dev*. 2008 Nov;22(22):3115–20.
- Milanovic M, Fan DN, Belenki D, Däbritz JH, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stemness. *Nature*. 2018 Jan;553(7686):96–100.
- Burd CE, Sorrentino JA, Clark KS, Darr DB, Krishnamurthy J, Deal AM, et al. Monitoring tumorigenesis and senescence in vivo with a p16(INK4a)-luciferase model. *Cell*. 2013 Jan;152(1–2):340–51.
- Hall BM, Balan V, Gleiberman AS, Strom E, Krasnov P, Virtuoso LP, et al. p16(Ink4a) and senescence-associated β -galactosidase can be induced in macrophages as part of a reversible response to physiological stimuli. *Aging (Albany NY)*. 2017 Aug;9(8):1867–84.
- Liu JY, Souroullas GP, Diekmann BO, Krishnamurthy J, Hall BM, Sorrentino JA, et al. Cells exhibiting strong p16 INK4a promoter activation in vivo display features of senescence. *Proc Natl Acad Sci USA*. 2019 Feb;116(7):2603–11.
- Childs BG, Baker DJ, Wijshake T, Conover CA, Campisi J, van Deursen JM. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science*. 2016 Oct;354(6311):472–7.
- Ueno M, Maeno T, Nishimura S, Ogata F, Masubuchi H, Hara K, et al. Alendronate inhalation ameliorates elastase-induced pulmonary emphysema in mice by induction of apoptosis of alveolar macrophages. *Nat Commun*. 2015 Mar;6(1):6332.
- Mikawa R, Suzuki Y, Baskoro H, Kanayama K, Sugimoto K, Sato T, et al. Elimination of p19ARF -expressing cells protects against pulmonary emphysema in mice. *Aging Cell*. 2018 Oct;17(5):e12827.
- Pathria P, Louis TL, Varner JA. Targeting Tumor-Associated Macrophages in Cancer. *Trends Immunol*. 2019 Apr;40(4):310–27.
- Kaneda MM, Messer KS, Ralainirina N, Li H, Leem CJ, Gorjestani S, et al. PI3K γ is a molecular switch that controls immune suppression. *Nature*. 2016 Nov;539(7629):437–42.
- Zhu Y, Herndon JM, Sojka DK, Kim KW, Knolhoff BL, Zuo C, et al. Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression. *Immunity*. 2017 Sep;47(3):597.
- Kaneda MM, Cappello P, Nguyen AV, Ralainirina N, Hardamon CR, Foubert P, et al. Macrophage PI3K γ Drives Pancreatic Ductal Adenocarcinoma Progression. *Cancer Discov*. 2016 Aug;6(8):870–85.
- Laberge RM, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L, et al. MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translocation. *Nat Cell Biol*. 2015 Aug;17(8):1049–61.
- Wislez M, Spencer ML, Izzo JG, Juroskie DM, Balhara K, Cody DD, et al. Inhibition of mammalian target of rapamycin reverses alveolar epithelial neoplasia induced by oncogenic K-ras. *Cancer Res*. 2005 Apr;65(8):3226–35.
- Sharpless NE, Ramsey MR, Balasubramanian P, Castrillon DH, DePinho RA. The differential impact of p16(INK4a) or p19(ARF) deficiency on cell growth and tumorigenesis. *Oncogene*. 2004 Jan;23(2):379–85.

- 32 Otterson GA, Kratzke RA, Coxon A, Kim YW, Kaye FJ. Absence of p16INK4 protein is restricted to the subset of lung cancer lines that retains wildtype RB. *Oncogene*. 1994 Nov;9(11):3375–8.
- 33 Okuma A, Hanyu A, Watanabe S, Hara E. p16Ink4a and p21Cip1/Waf1 promote tumour growth by enhancing myeloid-derived suppressor cells chemotaxis. *Nat Commun*. 2017 Dec;8(1):2050.
- 34 Jeon OH, Kim C, Laberge RM, Demaria M, Rathod S, Vasserot AP, et al. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat Med*. 2017 Jun;23(6):775–81.
- 35 Bussian TJ, Aziz A, Meyer CF, Swenson BL, van Deursen JM, Baker DJ. Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. *Nature*. 2018 Oct;562(7728):578–82.
- 36 Childs BG, Gluscevic M, Baker DJ, Laberge RM, Marquess D, Dananberg J, et al. Senescent cells: an emerging target for diseases of ageing. *Nat Rev Drug Discov*. 2017 Oct;16(10):718–35.
- 37 Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med*. 2016 Jan;22(1):78–83.
- 38 Adams JM, Cory S. The BCL-2 arbiters of apoptosis and their growing role as cancer targets. *Cell Death Differ*. 2018 Jan;25(1):27–36.
- 39 Baar MP, Brandt RM, Putavet DA, Klein JD, Derks KW, Bourgeois BR, et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell*. 2017 Mar;169(1):132–147. e16.
- 40 Fuhrmann-Stroissnigg H, Ling YY, Zhao J, McGowan SJ, Zhu Y, Brooks RW, et al. Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun*. 2017 Sep;8(1):422.
- 41 Sieben CJ, Sturmlechner I, van de Sluis B, van Deursen JM. Two-Step Senescence-Focused Cancer Therapies. *Trends Cell Biol*. 2018 Sep;28(9):723–37.