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Review

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Senescent T cells within suppressive tumor microenvironments: emerging target for tumor immunotherapy

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The functional state of the preexisting T cells in the tumor microenvironment is a key determinant for effective antitumor immunity and immunotherapy. Increasing evidence suggests that immunosenescence is an important state of T cell dysfunction that is distinct from exhaustion, a key strategy used by malignant tumors to evade immune surveillance and sustain the suppressive tumor microenvironment. Here, we discuss the phenotypic and functional characteristics of senescent T cells and their role in human cancers. We also explore the possible mechanisms and signaling pathways responsible for induction of T cell senescence by malignant tumors, and then discuss potential strategies to prevent and/or reverse senescence in tumor-specific T cells. A better understanding of these critical issues should provide novel strategies to enhance cancer immunotherapy.

Introduction

Cell senescence has been well recognized over the past several decades as a biological process with stable cell cycle arrest in diploid cells. This phenomenon was initially described in primary human fibroblasts after limited passages in cell culture (1). It has been shown that senescence can occur in various types of cells and tissues under different physiological and pathological conditions, including in normal aging, cancer, and infectious diseases (2–8). Senescent cells have permanent cell cycle arrest, but remain viable and metabolically active and possess unique functions and regulatory mechanisms that distinguish them from quiescent and apoptotic cells (9–12). Senescence induction in tumor cells directly controls tumor initiation, stemness, development, and proliferation via regulation of many oncogenes and the key cell cycle checkpoint genes (3, 13–17). In addition, induction of tumor cells to become senescent cells is a potential anticancer therapeutic strategy (13, 18, 19).

Recent studies have shown that senescence also occurs in human T cells, causing dysregulation of the immune system during the normal aging process (12, 20, 21). Furthermore, accumulation of senescent CD8⁺ T cells has also been found in younger patients with chronic viral infections, as well as patients with certain types of cancers (22–28). To explore the mechanisms responsible for the induction of senescent T cells in cancer patients, more recent studies suggest that both naturally occurring regulatory T cells (nTregs) and tumor-derived Tregs can strongly suppress naive/effector T cells through the induction of responder T cell senescence (29–32). In addition, different types of tumor cells can directly convert normal

immune cells into senescent T cells (27, 33, 34). These senescent T cells have altered phenotypes and possess strong suppressive activity that can potentially amplify immune suppression within the tumor microenvironment. Senescent T cells influence both immune cells and tumor cells through different potential molecular processes in the tumor microenvironment to promote tumor development and progression (discussed further in the following sections) (27, 29, 30, 33, 34). In addition, in vivo studies using adoptive transfer immunotherapy melanoma models have demonstrated that human tumor cells or Tregs can induce senescence in adoptively transferred tumor-specific T cells and decrease their antitumor efficacy (31–33). Notably, the incidence and prevalence of cancer are also markedly increased with aging, which could be due to the increase of senescence in T cells in elderly individuals (35–37). The increasing evidence clearly suggests that prevention of senescence development in effector T cells is urgently needed for successful tumor immunity and immunotherapy.

In addition to senescence in T cells, T cell exhaustion is another important dysfunctional state in cancers (38, 39). Senescent and exhausted T cells both have defective effector functions for tumor immunity, but they have distinct phenotypes and distinct regulatory mechanisms underlying their development and impaired antitumor functions (29–31, 40, 41). Exhausted T cells highly express a panel of inhibitory receptors, including programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), T cell immunoglobulin and mucin domain containing-3 (Tim-3), lymphocyte activation gene 3 (LAG-3), CD244 (2B4), and CD160 (42–47), and have been identified in patients with chronic viral infections and various types of cancers. Furthermore, exhausted T cells cannot proliferate, partially because of the PD-1-mediated suppression of T cell receptor (TCR) signaling (48). Exhausted T cells also display an impaired cytotoxic ability and production of effector cytokines such as

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Table 1. Senescent T cells in the tumor microenvironments

Tumor type	Cell subsets	Sites	Key results	References
Lung cancer	CD8 ⁺ CD28 ⁻	1.) Peripheral blood. 2.) Satellite lymph nodes and tumor lesions.	1.) Lung cancer patients show an expansion of the CD8 ⁺ CD28 ⁻ T cell subset in the peripheral blood, and these cells express high levels of Foxp3 and release IL-10 and TGF-β, exhibiting as CD8 ⁺ Treg phenotypes. 2.) The percentage of CD8 ⁺ CD28 ⁻ Tregs is increased in NSCLC patients and correlated with the pathological stages and tumor burden in patients. 3.) CD8 ⁺ CD28 ⁻ T lymphocytes constantly present in human tumors and inhibit effector T cell proliferative and cytotoxic functions.	22 54 53
Breast cancer	CD8 ⁺ CD28 ⁻ ; CD4 ⁺ CD28 ⁻ ; CD28 ⁻ CD57 ⁺ CD8 ⁺ ; CD28 ⁻ CD3 ⁺	1.) Peripheral blood. 2.) Sentinel nodes. 3.) TILs. 4.) Satellite lymph nodes and tumor lesions.	1.) Peripheral CD8 ⁺ T cells show downregulation of CD28 in cancer patients. 2.) The expression of CD28 and CD3-ζ in sentinel lymph nodes of breast cancer patients is decreased. 3.) The proportion of CD28 ⁻ CD57 ⁺ CD8 ⁺ T cells remains high among patients with cancer even after chemotherapy. 4.) Progressive elevated levels of CD8 ⁺ CD28 ⁻ suppressor T cells in metastatic breast cancer patients are a significant predictor for outcomes. 5.) CD3 ⁺ TILs derived from patients downregulate CD28 expression. 6.) CD8 ⁺ CD28 ⁻ T lymphocytes with suppressive activities exist in human tumors.	60 28 61 62 33 53
Ovarian cancer	CD8 ⁺ CD103 ⁺ CD28 ⁻ ; CD8 ⁺ CD28 ⁻	1.) Malignant ascites. 2.) Peripheral blood. 3.) Satellite lymph nodes and tumor lesions.	1.) Patients with advanced serous ovarian cancer have elevated frequencies of CD8 ⁺ CD103 ⁺ CD28 ⁻ T cells. 2.) The levels of CD8 ⁺ CD28 ⁻ T cells are high in patients and are correlated with the tumor burden and pathological stages. 3.) CD8 ⁺ CD28 ⁻ T cells in tumors suppress effector T cell proliferative and cytotoxic functions.	57 58 53
Head and neck cancer	CD8 ⁺ CD28 ⁻	1.) Peripheral blood. 2.) Satellite lymph nodes and tumor lesions.	1.) In patients with head and neck cancer, the frequency of the effector CD8 ⁺ CD28 ⁻ T cell population is increased while that of naive CD8 ⁺ CD28 ⁺ CD45RO ⁻ cells is decreased; and CD8 ⁺ CD28 ⁻ T cells are the apoptosis-sensitive subset and are terminally differentiated effector cells. 2.) CD8 ⁺ CD28 ⁻ T cells in tumors suppress effector T cell proliferative and cytotoxic functions.	23 53
Melanoma	CD8 ⁺ CD28 ⁻ CD27 ⁻ ; CD28 ⁻ CD3 ⁺ ; CD8 ⁺ CD28 ⁻	1.) Peripheral blood. 2.) TILs. 3.) Satellite lymph nodes and tumor lesions.	1.) CD28 ⁻ CD27 ⁻ CD8 ⁺ T cell subset is expanded in patients, which represents terminally differentiated effector cells expressing CD244 and high levels of perforin. 2.) TILs derived from patients downregulate CD28 expression. 3.) CD8 ⁺ CD28 ⁻ T cells in tumors suppress effector T cell proliferative and cytotoxic functions.	63 33 53
Multiple myeloma	CD57 ⁺ CD28 ⁻ CD8 ⁺ ; KLRG-1 ⁺ CD57 ⁺ CD160 ⁺ CD28 ⁻	1.) Peripheral blood. 2.) Bone marrow.	1.) T cells in MM patients display features of senescence at the tumor site, expressing CD57 but lacking CD28. 2.) Dysfunctional clonal T cells in MM exhibit a senescence phenotype, KLRG-1 ⁺ CD57 ⁺ CD160 ⁺ CD28 ⁻ , but with low PD-1 and CTLA-4 expression.	64 65
Colorectal carcinomas	CD27 ⁺ CD28 ⁻ CD8 ⁺ ; CD27 ⁺ CD28 ⁻ CD8 ⁺ ; CD8 ⁺ CD28 ⁻	1.) Peripheral blood and TILs. 2.) Satellite lymph nodes and tumor lesions.	1.) CD8 ⁺ TILs isolated from colorectal cancer patients are mainly CD27 ⁺ CD28 ⁻ or CD27 ⁻ CD28 ⁻ cells, which have low levels of perforin. 2.) CD8 ⁺ CD28 ⁻ T cell populations exist in human tumors and suppress effector T cells.	55 53
Endometrial carcinoma	CD8 ⁺ CD28 ⁻	1.) Peripheral blood. 2.) TILs.	The CD8 ⁺ T cells from PBLs and TILs express CD28, CD45RA, and CD45RO, but most tumor-infiltrating CD8 ⁺ T cells are CD28 ⁻ CD45RA ⁻ CD45RO ⁺ CCR7 ⁻ , suggesting a good terminal differentiation.	56
Cutaneous T cell lymphoma	CD8 ⁺ CD28 ⁻	1.) Peripheral blood. 2.) Skin infiltrates.	Increased percentages of CD8 ⁺ CD28 ⁻ suppressor lymphocytes in peripheral blood and skin infiltrates are correlated with advanced disease in patients with cutaneous T cell lymphomas.	59
Others: gastric, pancreatic, kidney, thyroid, esophageal, prostate, and neuroendocrine cancers	CD8 ⁺ CD28 ⁻	1.) Satellite lymph nodes. 2.) Peripheral blood. 3.) Tumor lesions.	CD8 ⁺ CD28 ⁻ T cell populations exist in human tumors and suppress effector T cells.	53

NSCLC, non-small cell lung cancer; PBL, peripheral blood lymphocyte; TIL, tumor-infiltrating lymphocyte.

IL-2, TNF, and IFN-γ (47). Unlike exhausted T cells, senescent T cells do not express increased levels of exhaustion-associated inhibitory molecules, but highly express senescence-associated β-galactosidase (SA-β-gal) and dramatically downregulate the costimulatory molecules CD27 and CD28 (7, 29–31, 49). Notably, senescent T cells have a unique senescence-associated secretory phenotype (SASP), producing high amounts of proinflammatory cytokines, which also is distinct from exhausted T cells (discussed in the following sections) (29–31, 33). Current

clinical trials using immune checkpoint blockade to interfere with CTLA-4 and/or PD-1/programmed cell death ligand 1 (PD-L1) have shown promising benefits for certain types of cancer patients, but overall success rates remain limited (50–52), suggesting that T cell exhaustion is not fully responsible for impaired antitumor function. Therefore, improved understanding of the molecular mechanisms involved in the induction and functional regulations of senescent T cells within the tumor microenvironment should lead to novel immunotherapies.

Table 2. Characteristics of senescent T cells

	Markers	Molecular features	References
The specific marker	SA- β -gal	High expression	29, 30, 49
Costimulatory molecules	CD27, CD28	Downregulation or loss of expression	29–31
Other associated molecules	Tim-3, CD57, KLRG-1	High expression	67–69, 71
Cell cycle arrest	Proliferation; p16, p21, and p53	Display cell cycle arrest and cannot proliferate with T cell receptor stimulation; high expression of p16, p21, and p53	29, 30, 33
Metabolic activation	SASP	Metabolically active; secrete high amounts of proinflammatory cytokines (IL-2, IL-6, IL-8, TNF, and IFN- γ) and suppressive cytokines IL-10 and TGF- β	29, 30, 33, 72, 73, 76, 77
Unique transcriptional profile	Transcriptional profile	Unique transcriptional profile distinct from that of anergic T cells, exhausted T cells, and quiescent terminally differentiated T cells	75–80
DNA damage	ATM, γ H2AX, p53BP	Upregulation of DNA damage response molecules	31, 32
Functional alterations	Killing abilities; negative regulatory functions	Defective killing abilities; negative regulatory functions; potent suppressive activity	25, 26, 74

ATM, ataxia-telangiectasia mutated; γ H2AX, phosphorylated H2AX; KLRG-1, killer cell lectin-like receptor subfamily G member 1; p53BP, p53-binding protein; SA- β -gal, senescence-associated β -galactosidase; SASP, senescence-associated secretory phenotype; Tim-3, T cell immunoglobulin and mucin domain containing-3.

T cell senescence is typical in suppressive tumor microenvironments

Substantial accumulation of senescent CD8⁺ T cells has been found among tumor-infiltrating lymphocytes (TILs) that are associated with various types of cancers, including lung (22, 53, 54), colorectal (55), endometrial (56), ovarian (57, 58), lymphoma (59), and breast cancers (28, 60–62), melanoma (33, 63), and multiple myeloma (MM) (64, 65), as well as with tumor metastases (22, 53). Recent studies have demonstrated that tumor-derived Tregs can induce T cell senescence (29–32). Furthermore, multiple types of tumor cells, including breast cancer, melanoma, colon cancer, prostate cancer, ovarian cancer, and head and neck cancer cells, can also directly induce T cell senescence (33, 34). These studies explain why senescent T cells accumulate within suppressive tumor microenvironments. Given that senescent T cells do not mediate antitumor activities, these observations strongly suggest that induction of T cell senescence is a key strategy used by malignant tumors to evade immune surveillance (refs. 12, 23, 29, 30, 33, 34 and Table 1).

Characteristics of senescent T cells

Senescence is an independent cell stage with unique phenotypes and functions. Unlike the extensive studies already focused on cell senescence in fibroblasts and tumors, very limited information is known about senescence in T cells (12). In recent studies of senescent T cells that develop during the normal aging process and in patients with chronic infections and cancers, senescent T cells display several specific characteristics (refs. 12, 22–30, and Table 2): (a) Senescent T cells highly express SA- β -gal (29, 30, 49). (b) Senescent T cells dramatically downregulate the costimulatory molecules CD27 and CD28 (7, 29, 66) and highly express other senescence-associated markers, including Tim-3, CD57, and killer cell lectin-like receptor subfamily G member 1 (KLRG-1) (67–71). (c) Senescent T cells upregulate cell cycle regulatory genes including p16, p21, and p53; are in a state of cell cycle arrest; and do not proliferate after TCR stimulation (29, 30, 33). (d) Senescent T cells remain metabolically active with a unique SASP (72, 73), producing high amounts of proinflammatory cytokines such as IL-2, IL-6, IL-8, TNF, and IFN- γ ,

as well as the suppressive cytokines IL-10 and TGF- β (29, 30, 33). (e) Senescent CD8⁺ T cells have defective killing abilities due to the loss of perforin and granzyme, or defects in granule exocytosis (7, 26). (f) Senescent CD8⁺ T cells have negative regulatory functions that reduce the protective effects of immunization, as well as prolong the survival of allografts (25, 74). (g) Senescent T cells themselves become suppressive cells mediating potent inhibition of proliferation and effector functions of other immune cells (29–31, 33). (h) Senescent T cells develop a unique transcriptional profile distinct from that of anergic T cells, exhausted T cells, and quiescent terminally differentiated T cells (31, 75–80).

Functional roles of senescent T cells in tumorigenesis

Increasing evidence strongly suggests that senescent T cells are critical mediators and amplifiers of immune suppression within the suppressive tumor microenvironment, promoting tumor development and progression (refs. 27, 29, 30, 33, 34 and Figure 1). A better understanding of the functional role of senescent T cells in tumor immunity is important for the development of novel cancer immunotherapeutic strategies.

T cell senescence is an important dysfunctional state with impaired antitumor capacities (12). In tumor microenvironments, senescent T cells are converted/differentiated from effector T cells and/or naive T cells. Once they become senescent, these cells are unable to respond to tumor antigen stimulation and recognition because of the downregulation of costimulatory molecules such as CD27 and CD28, the upregulation of inhibitory molecules including Tim-3 (7, 29, 67–71), and decreased production of the effector molecules perforin and granzyme (7, 26). Senescent T cells also actively suppress other immune cells within the tumor microenvironment. Senescent T cells induced by Tregs and tumor cells themselves become potent suppressor cells, directly inhibiting different types of immune cells, including Th1, Th17, CD8⁺ T cells, and DCs (29–31, 33). Furthermore, senescent T cells also secrete large amounts of IL-10 and TGF- β 1, inducing adaptive Tregs and increasing the immunosuppressive tumor microenvironments (29–31, 33).

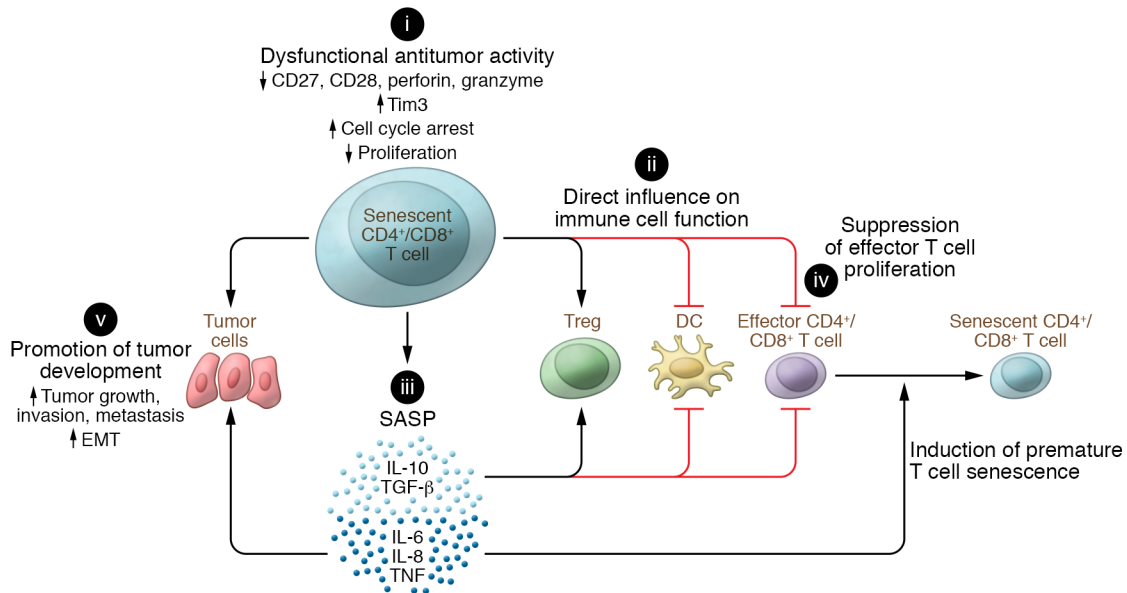


Figure 1. Effects of senescent T cells on tumorigenesis and cancer progression. (i) Senescent T cells have unique phenotypes with impaired antitumor activities. They downregulate the costimulatory molecules CD27 and CD28 as well as the effector molecules perforin and granzyme, and decrease proliferation, but promote cell cycle arrest and expression of molecules that inhibit proliferation. (ii) Senescent T cells can actively influence other immune cells within the tumor microenvironment. In addition to inducing adaptive Tregs, they can become potent suppressor cells themselves, performing direct inhibition on DCs and effector T cells. (iii) Senescent T cells have a unique SASP, secreting large amounts of cytokines that can induce premature senescence in T cells (IL-6, IL-8, TNF) and suppress effector immune cells (IL-10, TGF-β), as well as disrupt normal mammary differentiation and promote malignant phenotypes and tumor cell growth. (iv) Senescent T cells with potent suppressive activity can directly suppress effector T cell proliferation and function. (v) Senescent T cells can promote tumor growth, invasion, metastasis, and epithelial-to-mesenchyme transition via SASP or cell-cell direct contact.

In contrast to the functional defects in antitumor immunity, senescent T cells are metabolically active and have a unique SASP, which influences both immune cells and tumor cells in the tumor microenvironment. Recent studies clearly indicate that senescent T cells induced by tumor cells and Tregs can secrete large amounts of the cytokines IL-6, IL-8, and TNF (29, 30, 33, 34). These proinflammatory cytokines are critical inducers of premature senescence via autocrine or paracrine mechanisms (73, 81–83), which could induce more senescent T cells within suppressive tumor microenvironments. Furthermore, SASP can induce expansion of FoxP3⁺ Tregs and CD11b⁺Ly6G^{hi} cell populations and enhance their suppressive activity on tumor immunity (84, 85). In addition to establishment of a suppressive tumor microenvironment, the senescent cell-mediated SASP can directly favor malignant tumor progression through various effects, including disruption of normal mammary differentiation (86), inducing malignant transformation (87, 88), enhancing proliferation and invasiveness of neoplastic epithelial cells, promoting epithelial-mesenchymal transition, and increasing aggressiveness of metastatic cancers (Figure 1 and refs. 84, 86, 88–90). Sustained tumor growth can, in turn, eventually overwhelm the host’s ability to eliminate cancer cells, tipping the balance in favor of malignant cancer progression.

Metabolic alterations drive T cell senescence within tumor microenvironments

Although increased senescent T cells have been observed in various types of cancer patients, the mechanisms and factors responsible for the induction of T cell senescence in the tumor microenvironment are still under investigation (Figure 2). It is clear that

replicative senescence (telomere-dependent senescence), which occurs due to telomere shortening and/or dysfunctional telomerase that trigger a classical DNA-damage response (91, 92), contributes to aging and age-related pathologies in vivo (93). Furthermore, replicative senescence in T cells (CD8⁺CD28^{null} T cells) also occurs in patients with chronic infections, such as cytomegalovirus, Epstein-Barr virus, hepatitis C virus, and HIV infections (4–7). Repeated antigenic stimulation during chronic inflammation and persistent infection (viral, bacterial, or parasitic) induces extensive pathogen-specific T cell proliferation and prolonged activation, resulting in loss of telomerase activity, telomere shortening and/or telomere erosion, and replicative senescence (94, 95). Recent studies have demonstrated that TILs have short telomeres, suggesting that reintroduction of telomerase into T cells could be a novel strategy for tumor immunotherapy (96–98). However, a potential causal relationship between chronic exposure to tumor-specific antigens and accumulated senescent T cells within the tumor microenvironment remains unclear.

Increasing evidence indicates that development of T cell senescence in cancer patients is induced by tumor microenvironmental factors and extrinsic forms of stress, such as oxidative stress, DNA damage, activation of certain oncogenes, and production of inflammatory cytokines and chemokines (10, 99–101). Metabolic reprogramming is one of the important causes of T cell premature senescence in the tumor microenvironment. Tumor-derived Tregs can promote the conversion of responder T cells into senescent T cells (29–31, 33, 34). Mechanistically, Tregs exhibit heightened glucose uptake, increased glycolysis, and accelerated glucose consumption, which reduce glucose available

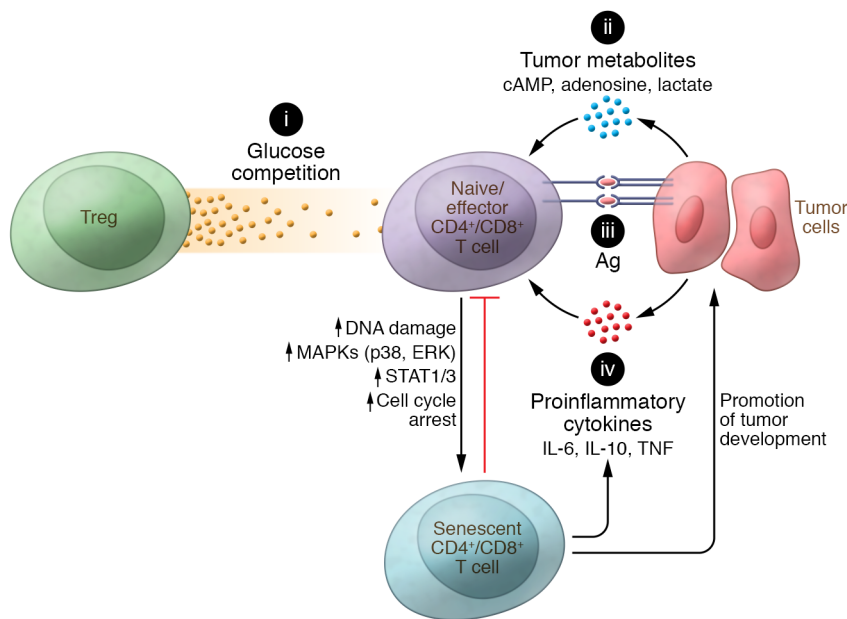


Figure 2. Potential signaling/mechanisms responsible for the development of senescent T cells within the suppressive tumor microenvironment. (i) Tregs' accelerated glucose consumption creates glucose competition in the tumor microenvironment, which can induce cell senescence in responder T cells during their crosstalk. (ii) Accumulation of metabolic end products, including cAMP, adenosine, and lactate, in the tumor microenvironment suppresses effector T cells and/or promotes induction of T cell senescence. (iii) Continuous and repeated stimulation from tumor antigens in T cells may induce loss of telomerase activity and result in replicative senescence of T cells. (iv) Tumor-derived microenvironmental inflammatory cytokines enhance tumor proliferation, inflammation, angiogenesis, and metastasis, and can also promote the development of senescent T cells. All these signaling pathways may potentially initiate the ATM-associated DNA damage response and activate MAPK and STAT1/3 signaling, resulting in T cell senescence in the tumor microenvironment.

for effector T cells. These interactions initiate activation of the AMP-activated protein kinase (AMPK) in responder T cells and eventually result in the DNA damage response associated with the nuclear kinase ataxia-telangiectasia mutated protein (ATM), and senescence in T cells (31, 32). It has been shown that low concentrations of glucose alone can significantly induce both CD4⁺ and CD8⁺ T cell senescence (31). In contrast, high concentrations of glucose (25 mM) dramatically prevent responder T cell senescence mediated by nTregs and tumor-derived Tregs (31, 32). Activation of AMPK, an important nutrient and energy sensor that is activated by reactive oxygen species and DNA-damaging agents, is the key step for T cell senescence (102–104). Activated AMPK can regulate cell cycle progression through increased phosphorylation of p53 and accumulation of p21^{WAF1} and p27 expression (105, 106). A recent study has shown that AMPK is activated in CD27⁺CD28⁺ senescent T cells, leading to autophosphorylation of p38 and inhibition of telomerase activity, as well as reduced T cell proliferation and expression of key components of the TCR signalosome (107). Furthermore, AMPK agonists can induce senescence characteristics in non-senescent T cells (107). Interestingly, tumor-infiltrating Tregs are highly activated and proliferative, and are not prone to senescence in the suppressive tumor microenvironment (31, 32, 108, 109). As discussed above, heightened glucose and lipid metabolism distinguishes both nTregs and tumor-derived Tregs from effector T cells (31, 32). Therefore, Tregs' energy demands, a combination of glycolysis and fatty acid synthesis and oxidation, confer a metabolic advantage that preferentially promotes proliferation and expansion in the tumor microenvironment (108). In addition, recent studies showed that Treg division and suppressive function are unaffected in the metabolically abnormal tumor microenvironment with low glucose and high lactate (109). Specifically, the transcription factor FoxP3 reprograms metabolism in Tregs, driving Treg resistance to the intracellular NAD depletion that results from the oxidation of L-lactate to pyruvate by lactate dehydrogenase; in contrast, intracellular NAD depletion dramatically impairs effector T cell function and proliferation (109). In

addition to tumor-derived Tregs, tumor cells themselves display heightened glucose and glutamine consumption, resulting in the depletion of nutrients and the accumulation of metabolites (110–112). More evidence suggested that tumor cells and TILs both compete for glucose within the suppressive tumor microenvironment, leading to cancer progression (113–115).

In addition to the direct competition for nutrients with effector T cells, accumulated metabolic end products, including cyclic adenosine monophosphate (cAMP), IDO, adenosine, and lactate, in the tumor microenvironment produced by tumor cells and Tregs are important inducers of T cell senescence (12, 32, 110–112, 116). cAMP is a potent inhibitor of effective tumor-specific T cells within the tumor microenvironment (111, 117). Furthermore, cAMP is also involved in Treg-mediated suppression (118). Studies have demonstrated that different types of tumor cells can directly induce conversion from naive/effector T cells into senescent T cells with potent suppressive activity (33, 34). High concentrations of endogenous cAMP exist in both tumor cells and tumor-induced senescent T cells and have been mechanistically implicated as responsible for the induction of T cell senescence. Tumor cells can transfer cAMP to targeted naive/effector T cells via gap junctions, resulting in markedly increased cAMP levels in senescent T cells. cAMP-induced T cell senescence is mechanistically dependent on the triggering of the ATM-associated DNA damage response in T cells (33, 119, 120). Unlike direct transfer of cAMP between cells, adenosine triggers immunosuppressive signaling via intracellular cAMP-elevating A_{2A} adenosine receptors on T cells (121). Chronic exposure of CD8⁺ T cells to exogenous adenosine can accelerate the process of cell senescence, causing reductions in overall proliferative potential and telomerase activity, and blunted IL-2 gene transcription (122). In addition, the loss of CD28 expression by senescent T cells is accelerated as a result of suppression of the CD28 promoter by adenosine-induced increases of caspase-3 (122). Notably, recent studies suggest that tumor-derived exosomes carrying numerous cargos, such as RNA and DNA, lipids, proteins, and metabolites, are also critical to regulate both tumor

cells and immune cells and to maintain a hypoxic and suppressive tumor microenvironment (123–126). These exosomes directly suppress antitumor activity and memory formation in effector T cells, as well as promote expansion and activation of Tregs (124, 127, 128). However, whether the tumor-derived exosomes also involve the development of T cell senescence in the tumor microenvironment remains unknown. A better understanding of the mechanistic links between tumor immunosuppression, hypoxia, metabolic dysregulation, and induction of T cell senescence should lead to novel strategies for cancer immunotherapy.

MAPK and STAT signaling critically control T cell senescence

It is well established that mitogen-activated protein kinase (MAPK) signaling is involved in controlling cellular senescence. However, the importance of MAPK signaling in T cell senescence was not explored until recently (12, 29, 31, 129, 130). ERK1/2 and p38 activation can induce p21-dependent G₁ cell cycle arrest as well as activate both the p53 and the pRb/p16 growth arrest pathways (131, 132). In addition, MAPK signaling pathways control oncogenic Ras-induced senescence (133–135). Furthermore, p38 MAPK is involved in regulation of many SASP cytokines and chemokines in senescent cells (136, 137). Constitutive p38 MAPK activation is sufficient to induce SASP, while inhibition of p38 MAPK signaling markedly reduces secretion of most SASP factors (138). More recent studies have shown that the CD27⁺CD28⁺ subset of CD4⁺ T cells exhibited elevated phosphorylation of p38, and that activation of p38 by AMPK and scaffold TAB1 induces human T cell senescence (129). Furthermore, the sestrin-dependent ERK-JNK-p38 MAPK activation complex controls T cell senescence (130). To identify the signaling pathways responsible for T cell senescence in the suppressive tumor microenvironment, recent studies have demonstrated that the cell cycle regulatory molecules p53, p21, and p16 are significantly increased in senescent T cells induced by both Tregs and tumor cells (29, 31, 33). Furthermore, ERK1/2 and p38 are selectively phosphorylated and activated during T cell senescence processes required for induction of responder T cell senescence mediated by human Tregs. In addition, STAT1 and STAT3 signaling mediates cellular senescence induced by H₂O₂, stress, and radiation (90, 139, 140). STAT1 and STAT3 signaling also involves T cell senescence mediated by both nTregs and tumor-derived Tregs (31). Collectively, ATM-associated DNA damage response, MAPK signaling, and STAT1 and STAT3 signaling cooperate to control T cell differentiation and development of senescence during the crosstalk with Tregs (Figure 2 and ref. 31). The studies discussed here and below identify the unique molecular signaling that controls and maintains T cell senescence in the tumor microenvironment, which should provide insights for the development of therapies designed to block T cell senescence and restore effector functions for tumor immunotherapy.

Senescence reversal and functional rejuvenation of tumor-specific T cells

All of these studies strongly indicate that malignant tumors use the induction of T cell senescence, which impairs antitumor capacities, as a strategy to escape the immune system (27, 29–34).

Therefore, developing strategies to prevent the generation of senescence and control the fate and function of tumor-specific T cells is critical for antitumor immunity.

Blocking molecular signaling important for senescent T cell differentiation. Inhibition of key signaling pathways controlling senescence induction in tumor-specific T cells could be a critical checkpoint for effective and enhanced antitumor immunity. Growth arrest in fibroblasts that are at an early stage of senescence can be reversed by blocking key cell cycle regulatory genes (p53, p38, and p21) and/or DNA damage response proteins (ATM or the cell cycle arrest protein CHK2) (92, 141–144). These studies have already provided critical insights for how to control the development of cell senescence. Recent studies have demonstrated that ATM signaling, MAPKs ERK1/2 and p38 signaling, and STAT1/STAT3 signaling are selectively activated during senescent T cell development induced by human Tregs and tumor cells (29, 31–33). Furthermore, blockage of ATM, ERK1/2, and p38 as well as STAT signaling pathways can considerably prevent the induction of T cell senescence in vitro and in vivo in animal models (29–31, 33, 34).

MAPK signaling is also critical for T cell activation and effector functions (144, 145). Therefore, selection of optimal MAPK inhibitors for preventing senescence induction without affecting proliferation and effector function in tumor-reactive T cells is urgently needed (12). MAPK inhibitors have been widely used in clinical trials for the treatment of melanoma patients (146–148), and some selective MAPK inhibitors have already shown significant enhancement of T cell recognition of melanoma without affecting lymphocyte function (149, 150). ATM inhibitors also have been used in clinical trials for cancer patients (151, 152). Those selective MAPK and ATM inhibitors already available for clinical use should be priority candidates for exploring the efficacy of T cell senescence inhibition in cancer patients (149, 150, 153, 154). In addition, recent studies have suggested that AMPK can trigger autophosphorylation of p38 and metabolic regulation of human T cell senescence during aging and interaction with Tregs (31, 107). Therefore, knockdown of AMPK by shRNA results in enhanced telomerase activity and proliferation of senescent T cells (107). Furthermore, sestrins bind to and coordinate ERK, JNK, and p38 MAPK activation in CD27⁺CD28⁺CD4⁺ T cells, and sestrin deficiency enhances T cell responsiveness and expansion in vivo during aging (130). Identification of more specific signaling pathways responsible for the generation of senescent T cells in the tumor microenvironment should lead to effective therapeutic targets for cancer immunotherapy.

Reprogramming Treg and tumor metabolism via TLR8 signaling. Both nTregs and tumor-derived Tregs can strongly suppress naive/effector T cells through the induction of responder T cell senescence, which mechanistically depends on glucose competition with responder T cells during their interaction (29, 30, 32). Toll-like receptors (TLRs) are very important for regulating Treg function (32, 155–158). TLR8 signaling reverses the suppressive functions of tumor-derived CD4⁺, CD8⁺, and $\gamma\delta$ Tregs, resulting in enhanced antitumor immunity mediated by tumor-specific CD8⁺ T cells in adoptive transfer tumor models (29, 30, 156, 157). Furthermore, TLR8 signaling in human Tregs can prevent their induction of senescence in responder T cells and DCs and can reverse the suppressor function of senescent T cells (29, 30).

Cellular energy metabolism also directs T cell survival, proliferation, and function (159–162), and both glucose and lipid metabolism is required for Treg suppression (32, 163). Recent studies suggest that TLR signaling directly regulates energy metabolism in immune cells, including in Tregs (164–166). TLR1 and TLR2 signaling activation in mouse Tregs increases Treg glycolysis and proliferation and reduces their suppressive capacity (167). Unlike TLR1/2 signaling in murine Tregs, TLR8 signaling activation can suppress both glucose uptake and transport as well as glycolysis in human Tregs, but does not alter glucose metabolism in naive and effector T cells, resulting in prevention of responder T cell senescence (31, 32). In addition to tumor-derived Tregs, tumor cells convert normal immune cells into senescent T cells via the metabolite cAMP, resulting in impaired antitumor immunity (27, 33, 34). Increasing evidence suggests that TLRs directly regulate metabolism, affecting tumor behavior and function in melanoma, prostate cancer, head and neck carcinoma, and breast cancer (33, 34, 158, 168). Human TLR8 signaling also directly targets multiple types of tumor cells and modulates the levels of endogenous metabolite cAMP in tumor cells, preventing their ability to induce T cell senescence (33, 34). Importantly, these *in vitro* studies were further confirmed *in vivo* for tumor immunotherapy, showing that TLR8 signaling can enhance antitumor immunity by preventing Treg- and tumor-induced senescence in tumor-specific effector T cells *in vivo* in the adoptive transfer therapy melanoma models (32, 33, 156, 157). These studies collectively indicate that human TLR8 signaling can reprogram glucose metabolism in both Tregs and tumor cells, resulting in suppression of their abilities to induce senescence in effector T cells. Therefore, TLR8 ligands could be effective tumor immunotherapeutic agents and/or adjuvants for tumor immunotherapy.

Concluding remarks and future perspectives

Current immunotherapies, including immune checkpoint blockade therapy and adoptive T cell therapy, have led to promising results in certain types of cancer patients, but the overall effective rates remain limited and vary among tumor types (51, 169). Exploration of alternative novel strategies targeting more specific checkpoint molecules or interrupting tolerogenic pathways is urgently needed. One of the key determinants of therapeutic efficacy is the functional state of the transferred/preexisting T cells in the suppressive tumor microenvironment (170, 171). Increasing evidence suggests that development of T cell senescence is a general feature and an important T cell dysfunctional state in the tumor microenvironment, which should be an emerging target for tumor immunotherapy (29–33). Therefore, preventing tumor-specific T cell senescence and rejuvenating effector T cell functions could become successful cancer immunotherapeutic strategies.

Cellular senescence has been recognized as a biological process over the past 50 years. However, the role and function of senescent T cells in tumor immunity remain unclear. Precisely dissecting the molecular mechanisms responsible for the development of T cell senescence will not only facilitate a better understanding of how malignant tumors escape immunity and sustain a suppressive tumor microenvironment, but also should provide emerging targets for tumor immunotherapy. Recent studies have identified MAPK and STAT3 signaling pathways as critical for controlling the

development of T cell senescence (29, 31). Furthermore, TLR8 signaling can reprogram metabolism in Tregs and tumor cells, reverse their suppressive effects, and prevent induction of T cell senescence mediated by tumors and Tregs (32, 33). Therefore, inhibition of MAPK signaling and/or TLR8 signaling activation should be effective strategies to control tumor-specific T cell senescence and dysfunction for tumor immunotherapy in the future.

It is now well recognized that the immune system can have both immune surveillance and tumor promotion effects during cancer development (172, 173). Furthermore, the functional role and subsets of T cells in the tumor microenvironment are dynamic during tumor progression (174). More efforts are needed to elucidate the alterations and plasticity among different states of T cells in the immunoeediting processes within the tumor microenvironment during cancer progression. In addition, different types of tumors are highly heterogeneous, associated with distinct escape mechanisms and clinical outcomes within T cell–inflamed and –non-inflamed tumor microenvironments (175, 176). T cell exhaustion and senescence are important dysfunctional states in cancers that are utilized by malignant tumors to escape antitumor immunity (29–31, 38–41). Therefore, a comprehensive understanding of the dynamic states and functions of T cells, including senescent and/or exhausted T cells in diverse tumor microenvironments, is essential for the development of novel therapeutic strategies to treat cancer patients. Recently, advancements in single-cell-based technologies and tissue imaging have made this possible (8, 177, 178).

Increasing evidence indicates that targeting T cell senescence is an emerging concept for enhancing tumor immunity and immunotherapy. Although great progress has been made in this specific area of cancer research, gaps in our understanding of the role of senescent T cells in different types of cancer patients remain. First, although induction of T cell senescence is a general feature within the suppressive tumor microenvironment, the causative mechanisms responsible for the senescent T cell development mediated by malignant tumors remain unclear. Cellular energy metabolism directs the fates and functions of T cells (159–162). However, the metabolic alterations involved in the induction and functions of these senescent immune cells in the tumor microenvironment are unknown. Therefore, improved understanding of metabolic regulations involved in the generation and development of senescent T cells mediated by different types of malignant tumors is urgently needed. These studies should lead to potential therapeutic targets for metabolic reprogramming to block senescence in tumor-specific T cells for cancer immunotherapy. Second, it is now recognized that senescent T cells are not exhausted and/or anergic T cells (31). Although senescent T cells are defective in antitumor immune functions, they are active and possess a unique SASP phenotype. SASP compositions include cytokines, lipids, metabolites, and extracellular vesicles and are complicated and highly heterogeneous, depending on duration of T cell senescence in the tumor progression (8, 179). However, the functional fractions of senescent T cell–derived SASP and its role in regulating effector immune cell functions and tumor development within the tumor microenvironment are unknown. Thus, further characterization of the unique molecular signatures and functional roles of SASP derived from senescent T cells during tumor development is needed to develop an effective and specific antitumor immunothera-

py. In addition, current clinical trials targeting CTLA-4 or PD-1/PD-L1 have yielded only limited success rates (51, 169). More recent studies have shown that PD-1-mediated suppression of T cell function results from dephosphorylation and inactivation of CD28 (180), and the CD28/B7 costimulatory pathway is required for effective anti-PD-1 therapy in cancer and chronic viral infection (181). Efficacy of anti-PD-1 therapy in cancer patients is related to PD-1⁺CD8⁺ T cell proliferation and the activation and expression of CD28 in PD-1⁺CD8⁺ T cells (181). One of the important characteristics of senescent T cells is downregulation or loss of CD28 expression (29–31). Therefore, it is difficult to activate senescent T cells in the tumor microenvironment.

Given that accumulated senescent T cells exist in cancer patients, the causative relationship between T cell senescence and unresponsiveness to current immunotherapies is another important but challenging issue that needs to be investigated. Compre-

hensively and precisely understanding these critical issues will provide insights for the development of promising clinical treatments against cancers in the future.

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