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Can senolysis be used to overcome tumor immune evasion?

Abstract

Tumor escape from immunologically mediated destruction is a well-studied phenomena and has been shown to utilize several pathways in common with physiological conditions such as pregnancy, as well as ocular or testicular immune privilege. Recent interest in senescence has revealed that senescent cells surrounding tumors contribute to development of a specific microenvironment that may allow for immune escape. Senescent cells have been reported to possess a "senescence associated secretory phenotype" (SASP) which produces inflammatory agents that directly and indirectly contribute to suppression of T cell and NK cell function. Exosomes secreted by senescent cells can suppress T cell activation, as well as downregulate activity of dendritic cells, which are needed for initiation of immunity. Studies have demonstrated that reduction of senescent cell load increases tumor sensitivity to a variety of therapies. We will overview supportive evidence for use of senolytics to potentiate the efficacy of immunotherapy in cancer, as well as discuss our preliminary findings regarding use of SenoVax™ (IND #30745), an autologous, polyvalent dendritic cell senolytic vaccine being developed for treatment of advanced non-small cell lung cancer.

Keywords: immune tolerance, cellular senescence, immunotherapy resistance, senolytics, T cell activation, NK cell function, tumor-associated immune suppression, senescence surveillance.

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Introduction

Since the birth of cancer immunotherapy with the work of William Coley at the beginning of the 20th Century,[1] our knowledge of the immune-tumor interaction has grown exponentially. Advances in the molecular and cellular understanding of tumor immunogenicity have led to identification of tumor-specific neoantigens, tumor immune evasion mechanisms, and therapeutic means of leveraging these discoveries. Although antigen specific immunotherapy has not yet lived up to its expected potential,[2] significant life extension of advanced cancer patients has been made possible by the introduction of checkpoint inhibitors.[3] According to some clinicians, the impact of this class of therapeutics has been described as a "revolution".[4] Another great success of immunotherapy was the development of Chimeric Antigen Receptor (CAR)-T cells which have elicited stunning successes in various hematologic malignancies.[5] Despite these accomplishments, there is still significant room for improvement. There remains a large proportion of patients who are somewhat resistant to checkpoint inhibitors, and thus development of treatment resistance represents a significant problem.[6,7] Resistance to immunotherapies is due in part to tumor associated immune suppression.[8,9]

Tumor evasion of endogenous immunity and immunotherapy has been studied in many situations and interestingly seems to leverage numerous mechanisms utilized by physiological conditions of immune tolerance such as pregnancy, as well as situations such as ocular or testicular immune privilege. For example, both pregnancy and cancer have been shown to utilize expression of indolamine 2,3 dioxygenase (IDO) to suppress T cell immune attacks.[10] The IDO enzyme suppresses immunity through depleting tryptophan, which is important in T cell proliferation/survival,[11] as well as producing immune modulatory metabolites such as kynurenine.[12] It is known that kynurenine increases T regulatory (Treg) generation[13,14] via activation of the aryl hydrocarbon receptor.[15] Other immune regulatory molecules such as HLA-G,[16] and TIM-3,[17] protect both tumor and fetus.

Other shared mechanisms include recruitment of immune suppressive cells such as gamma delta T cells,[18] myeloid suppressor cells,[19,20] and alternatively activated macrophages.[21] Additional similarities between cancer and immune privileged tissues include expression of T cell killing molecules such as Fas ligand, for which studies have shown the expression of this potent killing molecule on tumors,[22–25] corneal epithelial cells of the eye,[26] and Sertoli cells from the testicle.[27] In all these cases, blockade of Fas ligand results in damage to the respective tissue.[28–30] Other mechanisms that cancers have common with immune privileged tissues for mediating immune escape include expression of cytokines such as IL-10,[31–34] and TGF-beta.[35,36]

Although extensive data has been gathered on the immune-suppressive molecules that cancer uses to evade immunity, clinical translation beyond classical checkpoint inhibitors like CTLA-4 and PD-L1 remains limited. One potential reason for this is the field's focus on tumor cells themselves, rather than the surrounding cells. Research has highlighted the critical role of the tumor microenvironment in facilitating immune escape. One of the major cells involved in sculpting the tumor microenvironment is the M2 polarized macrophages. These cells possess an immune suppressive profile mediated by both contact dependent and independent mechanisms and have been demonstrated to block parts of the immune system that are involved in tumor destruction such as Th1 cells, cytotoxic T cells, and natural killer (NK) cells. The clinical relevance of these cells is demonstrated by numerous studies showing infiltration of neoplasia by M2 macrophages associated with poor prognosis in many of the major cancers including breast,[37] prostate,[38] lung,[39] melanoma,[40] and colorectal.[41] Conversely, infiltration of tumors with immune cells whose physiological role is eradication of cancer, such as NK cells, T cells, or activated dendritic cells is associated with better prognosis in numerous tumor types. Additionally, correlation has also been shown between the presence of "tumor eradicating" immune cells and response to immunotherapy and in some cases, chemotherapy as well.

Cellular senescence

Senescence occurs when cells reach a replicative limit or are exposed to various stressors. This causes cell cycle arrest, transformation into a flattened phenotype, and resistant to mitogens or proliferative signals while maintaining viability.[42] The concept of this "living/dead" cells was originally proposed by Hayflick,[43] after whom the term "Hayflick limit" was coined, which is the observation that generally cells undergo senescence after 50 doublings.[44] Senescence is believed to be mediated by telomere shortening, which could be overcome be the enzyme telomerase. For this paradigm shift, the first definitive understanding of the biology of aging, and arguably how to reverse it, the Nobel Prize was granted to Elizabeth Blackburn, Carol Greider and Jack Szostak in 2009.[45]

Senescence is associated with activation of various genes that also have tumor suppressor functions.[46] There is a belief that the process of senescence induction is one way by which cells avoid oncogenesis: if they are mitotically inactivated, they cannot become tumors. While originally, it was thought that senescent cells simply reside in an inert state, it was shown subsequently to not be the complete story. Senescent cells from a variety of tissues actively secrete inflammatory signals, something broadly termed "senescence associated secretory phenotype" (SASP). While these vary from cell to cell, SASP is associated with production of factors that potently modulate immunity. Classically, SASP was originally defined as production of agents such as: IL-1 beta, IL-6, and TGF-beta.[47] The defined and undefined factors produced by senescent cells appear to be responsible for the observations that these aged cells can actively transfer the senescent phenotype. For example, studies show that administration of a small number of senescent cells can accelerate aging in an otherwise healthy middle-aged mouse as shown by reduced function of numerous organs in an "infectious senescence" type manner.[48,49]

The 'senescence world view' has revolutionized our understanding of biological systems. Remarkably, senescence appears to play a role in nearly every disease process, ranging from COVID-19 pathology,[50] to post-infarct repair[51] and brain injury/ischemia,[52] and even transplant rejection. Overall, many conditions previously classified as 'inflammatory-mediated' now seem to have some involvement with the senescence pathways.

Given the old adage that cancer is the "wound that doesn't heal", it would be a logical step to look at the involvement of senescence in cancer.

Senescent cells in cancer and biological consequences

Studies have shown that oncogenes themselves can induce senescence in tumor cells, suggesting that the senescence mechanisms play a role in suppression of cancers. For example, Kang et al. utilized a model of oncogene induced liver cancer to show that the process of oncogene activation leads to "premalignant" senescence of hepatocytes. These hepatocytes were rapidly cleared in an antigen specific manner by Th1 cells in a macrophage dependent manner. If the T cells were inhibited, the mice would progress to full blown liver cancer.[53] In another study, it was found that senescence inducing agents such as aurora kinase inhibitors sensitize melanoma cells to T cell mediated killing by induction of senescence in the target cells.[54] Other examples of senescent cancer cells being more immunogenic have been described.[55] This is one example of "senescence surveillance" in which the tumor itself increases immunogenicity upon transformation to a senescent cell. Thus, cancer itself becoming senescent may be considered a good thing in some ways.

Cancer cells can also become senescent as a result of cancer therapy. It is widely documented that radiation therapy increases the proportion of senescent cells in tumors in vivo.[56] This is due in part because of oxidative stress, mitochondrial dysfunction, and DNA damage caused by radiation therapy, which induces stress-associated senescence.[57,58] In line with findings that senescence can increase immunogenicity of cancer cells, part of the radiation-induced abscopal effect has been attributed to radiation induced senescence.[59] Increase in senescent cells within tumors can also be seen after administration of chemotherapy.[60] This "therapy induced senescent cell" process plays an active role in adverse effects elicited by standard cancer therapies and in some cases reduction of these side effects can be seen when senolytic agents are administered. These pathologies consist of most of the dose-limiting factors associated with cancer therapeutics, such as cardiotoxicity,[50,61–65] neurotoxicity,[66–72] lung fibrosis,[73–78] and hematopoietic suppression.[79,80] This suggests that senolytics could play a game-changing role not only in potentially increasing efficacy of cancer therapeutics, but also reducing their side effects.

How do senescent cells evoke such a diverse range of pathologies? One mechanism is the active production of detrimental soluble factors. We previously mentioned that senescent cells via the SASP phenotype produce a variety of inflammatory cytokines and chemokines. It is believed that these, in part, contribute to the ability of senescent cells to expand their "pathology" to other cells. We know this to be the case based on several examples. In a study using clinical samples, cardiac progenitor cells were shown to have a senescent phenotype, and when cultured with non-senescent cells they could transfer this "dysfunction".[81] The classical SASP proteins are known to directly evoke inflammation, as well as indirectly recruit inflammatory cells. For example, interleukin-6 (IL-6) activates a plethora of inflammatory cytokines through its ability to activate the transcription factors STAT3 and NF-kappa B in both immune and non-immune cells.[82] Direct effects of IL-6 include enhancing endothelial activation to permit recruitment of inflammatory cells, suppression of neutrophil apoptosis to allow perpetuation of inflammation, and activation of monocytes. Additionally, IL-6 is involved in generation of Th17 cells, which possess numerous downstream inflammatory activities and have been associated with numerous age-associated inflammatory conditions. The clinical importance of IL-6 is that blocking this inflammatory cytokine has demonstrated some clinical efficacy in conditions such as rheumatoid arthritis, COVID-19,[83] and immunotherapy induced cytokine storm. Other SASP proteins such as IL-1 alpha, IL-1 beta, and IL-8 all possess ability to induce cellular stress through stimulation of inflammatory cascades. These cascades have the ability to cause numerous detrimental reactions that damage cells in an almost organ-nonspecific manner.

Senescent cells surrounding cancer

One of the major realizations in the development of oncology therapeutics is that cancer cells, as we know them based on laboratory growth, do not represent cancer cells growing in vivo. This likely explains the overall difficulty in translating laboratory findings to the clinic. In vivo tumors rely on numerous cells for their growth and metastasis. For example, cancers need external endothelial cells for generation of angiogenesis, without which cancers cannot grow more than 2 mm. Tumors leverage fibroblasts to achieve a growth advantage and escape from immunity, and as discussed above, tumors also leverage macrophages for angiogenesis and immune modulation.

What is becoming increasingly apparent is that many of the cell surrounding the tumor seem to possess SASP. Could these cells be responsible for immune evasion? What is the best way of clearing them?

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The clearance of senescence cells in vivo has been pioneered by the laboratory of James Kirkland who used a computer-based approach to identify biological pathways associated with senescence and subsequently chose clinically applicable means of blocking them.[84] They found the combination of the polyphenol quercitin and dasatinib to be the most potent. They also demonstrated that this combination can be used clinically, which reduced levels of senescent cells.[85]

Since killing of senescent cells is possible in vivo, what happens in tumor models when one inactivates senescent cells? Induction of senolysis makes tumors become more sensitive to killing by radiation,[86–91] chemotherapy,[92–100] and immunotherapy.[101–103] Assouline et al. found that senescent stromal cells surrounding pancreatic tumors in mice and humans possessed significantly higher levels of immune suppressive cytokines. In pre-clinical/animal cancer models, depletion of senescence cells using a gene-based system or by administration of small molecules which block the b cell lymphoma (bcl)-2 gene, resulted in a change in the tumor microenvironment from "immune suppressive" to "immune stimulatory." This was characterized by increased cytotoxic T cell infiltration. Most clinically relevant, the reduction of senescent cells resulted in increased sensitivity of pancreatic cancer cells to checkpoint inhibitors.[104] In the case of breast cancer, Stewart et al. identified that stromal cells surrounding the tumor express a high degree of senescence. They secrete immune suppressive cytokines and an immune inhibitory extracellular matrix. Killing the cancer associated senescent cells using two independent means "removed the tumor's shield" and allowed for immune mediated tumor regression. Importantly, these "immune suppressant" stromal cells were found in various stages of breast cancer and predicted for tumor recurrence.[105]

Senescent cells within and surrounding tumors play a role in protecting it from immune attack, as well as promoting angiogenesis, metastasis and drug resistance. More troubling is the fact that the vast majority of our current cancer treatments, instead of reducing senescent cells, increase them, potentially increasing the aggressiveness of the tumor. Although numerous senolytic molecules have been reported, a significant drawback is potential off-target toxicity.

The natural role of the immune system in suppressing senescence

While conventionally we think of the immune system as playing a role in host defense against pathogens, various aspects of the immune system are needed for maintaining physiological functions and "clearing" unwanted tissues. For example, the complement part of the immune system is involved in pruning of neurons during brain development.[106] Evidence of the fundamental role of this process is that mice lacking C1q develop excessive neurons due to impaired pruning and as a result suffer from epilepsy.[107] Another example is how a unique type of antibody, whose receptors are germline encoded, called "natural antibodies" play a fundamental role in clearing various types of injured tissues so as to prevent fibrosis and reduce the possibility of nonfunctional tissue from interfering with proper organ functions.[108–110] Accordingly, the possibility that the immune system plays a role in preventing accumulation of senescent cells has been investigated.

One of the first unequivocal demonstrations of the importance of immunity in clearing senescent cells was a study by Xue et al. in which reactivation of p53 in hepatic neoplasia resulted in senescence induction. This was accompanied by clearance of the newly generated senescent cells by neutrophils and NK cells.[111] The ability of CD8 T cytotoxic cells to kill senescent cells has been demonstrated in a series of experiments showing that senescence induces an increase in antigen presentation by MHC I, as well as secondary signals, or inducers of secondary signals, such as alarmins.[112] Senescent cell activation of CD4 T cells by MHC II has been demonstrated in primary human melanocytes in which NRAS Q61K and BRAF V600E induced senescence was shown to elicit augmented MHC II, increased proteins associated with antigen presentation, and stimulation of the MHC II upstream factor CIITA.[113]

Feasibility of inducing immunity to senescent cells was demonstrated by Suda et al, who identified that antigen protein glycoprotein nonmetastatic melanoma protein B (GPNMB) is selectively found on senescent cells. They induced immunity to GPNMB through standard vaccination and showed a profound reduction of senescent cells in vivo, as well as improved function in older mice, in some ways, more potent than achieved with small molecule senolytics.[113] Feasibility of breaking tolerance to senescent cells and induction of T cell dependent senolysis has been replicated by other groups.[114]

Another alternative strategy is to generate "supercharged" T cells that selectively only kill senescent cells. To accomplish this, investigators have applied the clinically successful CAR-T approach. This approach is exciting because it has been shown that these gene-engineered cells possess selectively the ability to act as "serial killers" and persist for up to ten years in people, generally without serious adverse effects.[114] With this in mind, Amor et al. generated CAR-T targeting of the senescence associated molecule urokinase-type plasminogen activator receptor (uPAR) and demonstrated in vitro and in vivo activity of these cells, including ability to induce tumor regression by ablating the peritumoral senescent cells.[115] A subsequent publication from the same group demonstrated that these senolytic CAR-T cells are capable of exhibiting actual "anti-aging" activities such as enhanced exercise capacity in healthy, but old mice and improved glucose control in aged and obese mice.[116]

SenoVax™: leveraging dendritic cells and polyvalent antigens for senolysis

Despite the previously mentioned progress in removing senescent cells by upregulation of immunity, there are potential drawbacks to current approaches. Vaccine approaches have limited ability to potently stimulate immunity. This may account for the relatively mediocre results of standard "antigen + adjuvant" vaccines. Additionally, single-antigen approaches leave open the possibility of immune editing and immune escape. CAR-T approaches are limited by the fact that they can only be used against a single antigen.

SenoVax™ is an autologous, polyvalent dendritic cell vaccine that utilizes in vitro generated senescent "stromal cells" as a source of antigens. From the antigenic source perspective, SenoVax™ is created by expansion of stromal cells obtained by punch biopsy from the patient's skin. These cells are expanded ex vivo under conditions that replicate tumor-associated microenvironment and senescence induction. SenoVax™ stromal cells are assessed for appropriate concentrations of senescent antigens and then prepared in a proprietary manner in order to allow them to be taken up by dendritic cells generated from peripheral blood mononuclear cells of the patient. These dendritic cells are then stimulated to mature and used as a "cellular immunotherapy."

In a patent filed by Immorta Bio, potent suppression of in vitro generated senescent fibroblast (Figure 1a) but not control fibroblast

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(Figure 1b) viability was observed. Furthermore, both a single injection of SenoVax (Figure 1c), as well as multiple injections (Figure 1d) resulted in inhibition of growth of Lewis Lung Carcinoma (LLC) tumors. Immorta Bio has filed an Investigational New Drug Application (IND #30745) with the plan of initiating a dose-escalating proof of concept clinical trial in advanced lung cancer patients.

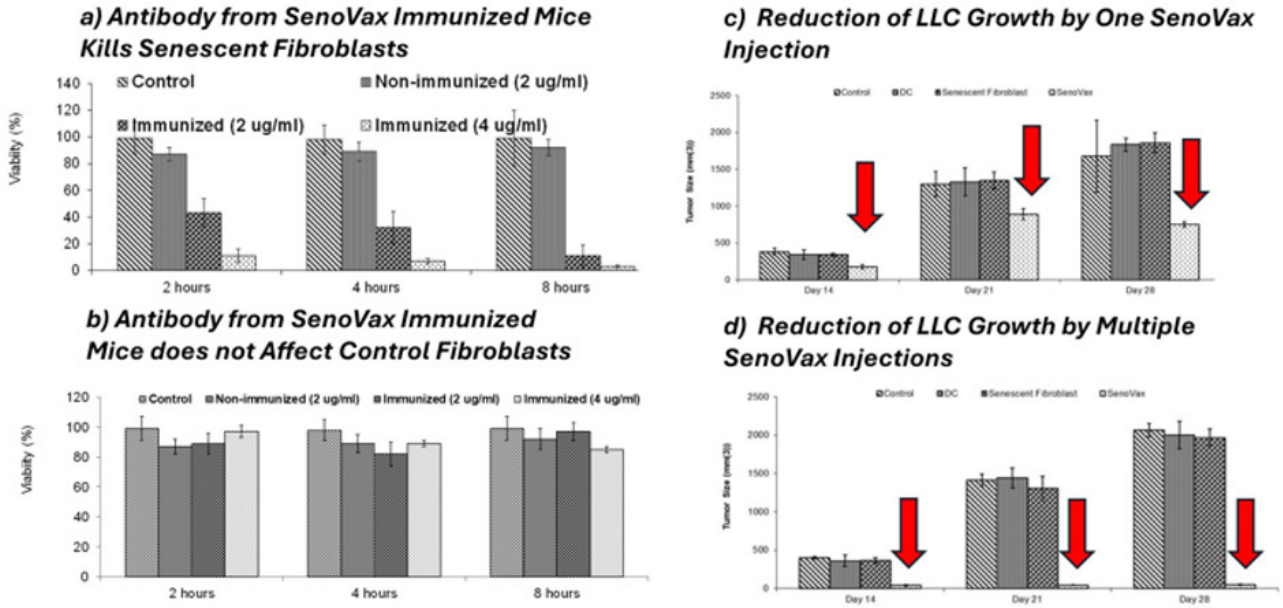

Figure 1 SenoVax™ antibody effects on senescent fibroblasts and tumor growth inhibition.

Female C57BL/6 mice of 6-8 weeks of age were subcutaneously implanted with 500,000 Lewis lung carcinoma cells (LLC) from ATCC. Mice were treated with one injection of the therapeutic agents: a) Saline; b) Syngeneic Dendritic Cells generated by 7-day culture (500,000), c) Senescent Fibroblast Extract; d) Dendritic Cells Pulsed with Senescent Fibroblast Extract (SenoVax). Tumors were measured by calipers and size was expressed as mm (3). For generation of dendritic cells, bone marrow mononuclear cells were flushed from the femurs and tibias of tolerant, rejective, and naive mice, washed, and cultured at 2 × 10^6 cells/well in 24-well plates (Corning Glass, Corning, NY) in 2 ml RPMI 1640 (Life Technologies, Ontario, Canada) supplemented with 10% FCS (Life Technologies), 100 U/ml of penicillin, 100 µg/ml of streptomycin, 50 µM of 2-ME (Life Technologies), 10 ng/ml of murine rGM-CSF (Peprotech, Rocky Hill, NJ), and 10 ng/ml of IL-4 (Peprotech). Nonadherent cells were removed after 48 h of culture, and fresh medium was added every 48 h. DC were used for in vitro experiments after 7 days of culture. Fibroblasts were obtained from skin biopsy of syngeneic mice and expanded in the presence of doxorubicin to induce a senescent phenotype. It is expected that this initial demonstration of polyvalent immunization against cancer-associated senescent cells will allow for future combination therapies

Conclusion

In conclusion, this paper highlights the complex role of cellular senescence in the tumor microenvironment, emphasizing how senescent cells contribute to immune evasion by possessing a "senescence associated secretory phenotype" (SASP) and secreting exosomes. Despite the challenges posed by the senescent cells in promoting tumor growth and resistance to therapy, emerging research shows that targeting these cells with senolytics can enhance the effectiveness of immunotherapies. Preliminary findings with SenoVax™, a dendritic cell-based senolytic vaccine, offer promising potential for treating advanced cancers by not only eliminating senescent cells but also boosting anti-tumor immunity. This approach represents a novel therapeutic avenue with the potential to transform cancer treatment by simultaneously addressing tumor cells and their supportive microenvironment.

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Conflicts of interest

Wally Veklych, Thomas E. Ichim, Robert Reznik, Vladyslav Bykoriz, Yuri Kaplun, and Boris N. Reznik are shareholders and management of Immorta Bio, Inc., a company holding patents and developing therapies for senescence.

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