RESEARCH HIGHLIGHT

Immune checkpoint inhibitors as senolytic agents

Parminder Singh[1], Pankaj Kapahi[1] and Jan M. van Deursen[2]✉

Cell Research (2023) 33:197–198; https://doi.org/10.1038/s41422-022-00761-4

Senolysis, the elimination of senescent cells (SNCs), is a novel therapeutic concept for the treatment of aging and age-related diseases that has scored a first success in the clinic for the treatment of diabetic macular edema with UBX1325. In a recent *Nature* paper, Wang et al. now report that subsets of SNCs express PD-L1 and describe a novel mode of senolysis through clearance of these cells by immune checkpoint inhibitors.

Cellular senescence is a cell fate program that protects mammals against cancer and promotes tissue repair and regeneration.[1] However, it also has a dark side in that senescent cells (SNCs) that accumulate in tissues and organs can be a cause of tissue degeneration and dysfunction. SNCs exert both their beneficial and detrimental biological effects through the senescence-associated secretory phenotype (SASP), a complex heterogenous secretome whose composition varies per cell type, senescence inducer, and senescence stage.[2]

Proof-of-concept studies in mice demonstrating that senolysis slows features of aging and age-related diseases[3] initiated a hunt for drugs that selectively and safely eliminate detrimental SNCs implicated in diseases such as atherosclerosis, osteoarthritis, pulmonary fibrosis, macular degeneration, dementia, and various other conditions. It turned out that the viability of pathology-causing SNCs is frequently dependent on the pro-survival functions of BCL family members, particularly BCL2 and BCL2L1,[4] with synthetic small-molecule inhibitors of these proteins having meaningful therapeutic effects in preclinical models of most of the abovementioned diseases. Although these inhibitors are not suitable for systemic use in humans because of on-target adverse effects on neutrophils and thrombocytes, they have been proven to be safe and highly effective upon intraorbital injection in patients with macular disease (NCT04857996).[5]

Naturally occurring flavonoids with senolytic properties such as quercetin (typically used in combination with the tyrosine kinase inhibitor dasatinib) and fisetin have also been widely tested in preclinical models.[6] Encouraging results in these studies together with a lack of overt adverse effects allowed these compounds to quickly advance into two dozen clinical trials for a wide variety of age-related disorders that require systemic administration. One key note of caution here is that the biological properties of fisetin, quercetin, and dasatinib are highly diverse, which will complicate the interpretation of any clinical effects in terms of senolysis. Furthermore, SNCs can be highly heterogeneous, and therefore multiple modes of clearing them may be required for future therapeutics. To further advance senolysis as a therapeutic concept, Wang and colleagues hypothesized that the immune checkpoint might play a central role in SNC survival, and if so, that checkpoint inhibitors might be attractive therapeutics for the treatment of age-related phenotypes (Fig. 1).[7]

An emerging idea in the field is that pathological SNCs are capable of accumulating over a long period in tissues and organs by escaping immune clearance.[8] One potential escape mechanism that has been explored by several laboratories involves the expression of immune checkpoint proteins by SNCs. Indeed, Onorati and colleagues recently reported that in vitro derived SNCs express high levels of *CD274*, the gene encoding the checkpoint protein PD-L1, regardless of cell type or senescence-inducing stressor.[9] They found PD-L1 upregulation to be a late event in the senescence program that is dependent on proinflammatory elements of the SASP. The new study by Wang and coworkers adds to that a dependence on low-level E2F transcriptional activity and impaired proteasome activity (Fig. 1).[7] The authors further show that PD-L1 upregulation protects in vitro SNCs against co-cultured activated T cells and that PD1-neutralizing antibodies abolish this protective effect, triggering the elimination of PD-L1$^+$ SNCs in a SASP- and antigen-dependent fashion (Fig. 1).

Using a previously established tdTomato-based reporter strain to mark p16$^+$ SNCs in mouse tissues and organs,[10] Wang et al. provide evidence to suggest that their in vitro findings are relevant in vivo, and that immune checkpoint inhibition might have therapeutic applications beyond cancer treatment. They observed that ~50% of the Tom$^+$ SNCs in aged livers are PD-L1$^+$, with lower percentages of these cells (~10%) also being observed in aged lungs and kidneys. These percentages markedly decrease in a CD8$^+$ T cell-dependent fashion when PD1-neutralizing antibodies are administered. Importantly, treatment of aged mice with PD1-neutralizing antibodies ameliorated several aging phenotypes including hepatic lipidosis, decreased grip strength and motor skills, and increased alveolar volume. Furthermore, in a mouse model for NASH, immune checkpoint inhibition slowed disease progression reminiscent of SNC elimination with the senolytic drug ABT263. Collectively, these data indicate that the elimination of PD-L1$^+$ SNCs via immune checkpoint inhibition could be a promising therapeutic anti-aging strategy.

However, the data reported also raise several questions that will require further analysis. While PD1-neutralizing antibodies eliminate a large proportion of PD-L1$^+$ Tom$^+$ cells, a smaller but substantial proportion of PD-L1$^-$ Tom$^+$ cells disappear as well. Because PD-L1$^-$ cells constitute the dominant Tom$^+$ population in all tissues analyzed, the absolute numbers of PD-L1$^-$ Tom$^+$ cells targeted for elimination with anti-PD1 treatment appear to be much larger than those of corresponding PD-L1$^+$ Tom$^+$ cells. This

[1]Buck Institute for Research on Aging, Novato, CA, USA. [2]Cavalry Biosciences, Novato and San Francisco, CA, USA. ✉email: janvandeursen2@gmail.com

Published online: 8 December 2022

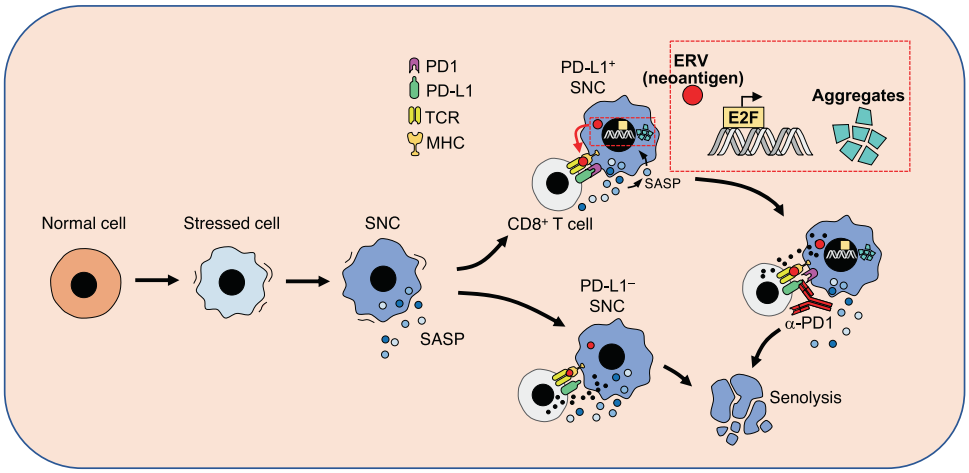

Fig. 1 Immune checkpoint activation in SNCs. Stressed or damaged cells in a senescent state remain cell cycle arrested and produce a secretome enriched in immune modulatory factors that attract immune cells, including cytotoxic T cells that kill their target. Some SNCs escape clearance by inducing the checkpoint protein PD-L1, an event that involves autocrine SASP signaling, E2F transcriptional activity, and impaired proteasome activity. This rescue mechanism can be overcome by the administration of immune checkpoint inhibitors.

raises interesting questions about the mechanism of clearance of PD-L1^{-} Tom^{+} cells and their contribution to the therapeutic effects observed with anti-PD1 treatment. Furthermore, these experiments do not rule out the importance of Tom^{-} cells that may also be targeted by the anti-PD1 treatment. Moreover, because only a subset of SNCs is p16^{+}, it remains to be explored to what extent p16^{-} SNCs upregulate PD-L1 and contribute to the therapeutic benefits of anti-PD1.

It will also be important to further investigate how aging-related phenotypes improve with treatment. For instance, with regards to grip strength, does improvement upon treatment correlate with changes in muscle tissue that would be consistent with reduced sarcopenia? Also, by including baseline values of aging-related phenotypes analyzed, one would be able to assess whether therapeutic effects represent a reduced rate of progression or a reversal of these phenotypes. And finally, anti-PD1 treatment may not resolve the risk for adverse effects that systemic administration of senolytic drugs poses through the elimination of beneficial SNC populations implicated in tissue repair or rejuvenation.

REFERENCES

1. van Deursen, J. M. *Nature* **509**, 439–446 (2014).
2. Coppe, J. P. et al. *PLoS Biol.* **6**, 2853–2868 (2008).
3. Baker, D. J. et al. *Nature* **479**, 232–236 (2011).
4. Chang, J. et al. *Nat. Med.* **22**, 78–83 (2016).
5. Crespo-Garcia, S. et al. *Cell Metab.* **33**, 818–832.e7 (2021).
6. Chaib, S., Tchkonia, T. & Kirkland, J. L. *Nat. Med.* **28**, 1556–1568 (2022).
7. Wang, T. W. et al. *Nature* **611**, 358–364 (2022).
8. Kale, A., Sharma, A., Stolzing, A., Desprez, P. Y. & Campisi, J. *Immun. Ageing* **17**, 16 (2020).
9. Onorati, A. et al. *Mol. Cell Biol.* **42**, 948–956 (2022).
10. Omori, S. et al. *Cell Metab.* **32**, 814–828.e6 (2020).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Jan M. van Deursen.