Immune cells engineered to target senescence

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The cellular state of senescence is a hallmark of ageing. It can be beneficial but is also implicated in some diseases. A method developed to harness immune cells to target senescent cells might offer new therapeutic possibilities.

Senescence is a type of cellular stress response. In some circumstances it is harmful if cells enter into a state of senescence, and efforts are ongoing to develop therapies that target senescent cells. Writing in *Nature*, Amor *et al.*[1] describe a method that selectively removes senescent cells from mice[2]. Entry into senescence imposes a stable arrest of the cell cycle, which prevents the division of old, damaged or precancerous cells. Senescent cells secrete a complex cocktail of factors, which drives what is called the senescence-associated secretory phenotype (SASP) response. This recruits T and NK cells of the immune system, which promote the removal of the senescent cells. Under these conditions, senescence is transient and it benefits the organism.

However, when senescent cells linger, they can promote chronic inflammation resulting in age-related diseases such as atherosclerosis, lung or liver fibrosis and cancer. The elimination of senescent cells has therefore emerged as a promising therapeutic strategy. It can improve the outcome of many diseases and increase lifespan in studies of mice[3]. One possible way to target senescent cells is the use of specific drugs, called senolytics, for this purpose Amor and colleagues take a different approach, getting inspiration from the observation that immune cells are involved in eliminating senescent cells under normal circumstances.

Amor *et al.* adapted a technique that is currently in use for anticancer treatment. In this therapy, a type of immune cell called a T cell is taken from a person and, before being returned to the body, the cells are manipulated to

boost their ability to target cancer cells. Such cells are known as CAR T cells because they are engineered to express what is termed a chimaeric antigen receptor (CAR). The CAR is designed to recognize a particular small fragment, called an antigen, of a protein present on the surface of cancer cells. If this interaction occurs, it activates the T cell to kill the tumour cells[1]. Identifying antigens that are expressed exclusively on tumour cells is a key challenge because the killing of healthy cells by CAR T cells could lead to severe side effects.

To find antigens that are specific for senescent cells, Amor and colleagues analyzed the expression of transmembrane proteins found in senescent human and mouse cells. One of the eight most-promising candidates identified was urokinase plasminogen activator receptor (uPAR). Examining previously published data of protein expression in human tissues revealed that uPAR is either not detected or present at low levels in most organs of the human body, including in the central nervous system, the heart and the liver, however, uPAR is highly expressed in different senescent cells *in vitro* and *in vivo.* Intriguingly, a soluble form of uPAR (suPAR) that lacks a transmembrane region is a component secreted during the SASP response. The presence of suPAR is a biomarker that is a hallmark of some chronic diseases, including diabetes and kidney disease, in which senescence has a role.

After identifying uPAR as a universal marker of senescent cells, Amor and colleagues engineered CAR T cells able to target uPAR (Fig. 1). Given that premalignant cells (those possibly on their way to become cancer cells) undergo senescence and the fact that many anticancer therapies work by causing tumour cells to enter senescence, the authors began by investigating how effective these CAR T cells were for treating cancer. Amor *et al*. report that treatment with CAR T cells that target uPAR eliminated senescent (pre)malignant cells in mouse models of liver and lung cancer. It has already been proposed that to improve the success of anticancer therapies, they should be followed up with treatments targeting senescent cells[2]. Amor and

colleagues' study of mice confirm that such an approach using their senolytic CAR T cells boosts the effectiveness of anticancer treatment***[OK?]***.

Part of the attraction of using senolytic CAR T cells is their potential to treat the many diseases in which senescence is involved. Indeed, Amor and colleagues show that senolytic CAR T cells improve the outcome of a type of tissue scarring known as liver fibrosis in mouse models of the condition non-alcoholic steatohepatitis compared to the case of the animals that did not receive these T cells.

The most widely used senolytic drug, Navitoclax can cause toxicity that limits its use. This has led to efforts to identify new senolytic drugs and other ways to target senescent cells[3]. Amor *et al*. suggest that senolytic CAR T cells could bypass some side effects and the limited effectiveness associated with senolytic drugs. However, CAR T cells are not necessarily problem-free. A common complication of the therapeutic use of CAR T cells is a condition called severe cytokine release syndrome (an outcome that is also described as a cytokine stor*m)*, in which an intense T-cell response causes fever and affects blood pressure and breathing. Although the authors observed that high doses of the senolytic CAR T cells caused cytokine release syndrome, if the dosage was reduced this avoided the problem whilst retaining the therapeutic potential of the treatment.

The use of CAR T cells for anticancer therapy has other limitations. Long-lasting activity of CAR T cells is required for effective tumour control as cancer cells divide over time. This issue might not be of concern when targeting senescent cells because they do not proliferate***.*** Many solid tumours (those that do not arise from blood cells) are associated with an immunosuppressive microenvironment, which can cause CAR T cells to enter a dysfunctional state called exhaustion***.*** Senescent cells can foster an immunosuppressive microenvironment during tumour formation[4]. Although the authors did not observe senescence-mediated immunosuppression in this study, in case it might be a shortcoming of this approach a greater understanding is needed of how senescent cells can interfere with immune-system function.

Will senolytic CAR T cells be used in the future to treat patients? Using CAR T cells in the clinic is expensive, so the criteria for which such an approach might be considered should be chosen carefully. It will also be important to determine whether CAR T cells that target human uPAR are as safe and effective as the CAR T cells targeting mouse uPAR that Amor and colleagues used. Or perhaps this method might move forward by using senolytic CAR T cells that target other proteins found on the surface of senescent cells such as DPP4 or oxidized vimentin[5]. The immense advances that are being made in mapping proteins expressed in the human body at the resolution of single cells might reveal additional targets for use in the design of senolytic CAR T cells. The merging of therapeutic strategies that use CAR T cells with the approach of targeting senescent cells might be a powerful combination to tackle certain diseases.

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1 Lim, W. A. & June, C. H. The Principles of Engineering Immune Cells to Treat Cancer. *Cell* **168**, 724-740, doi:10.1016/j.cell.2017.01.016 (2017).
2 Wang, L. & Bernards, R. Taking advantage of drug resistance, a new approach in the war on cancer. *Front Med* **12**, 490-495, doi:10.1007/s11684-018-0647-7 (2018).
3 Ovadya, Y. & Krizhanovsky, V. Strategies targeting cellular senescence. *J Clin Invest* **128**, 1247-1254, doi:10.1172/JCI95149 (2018).
4 Eggert, T. *et al.* Distinct Functions of Senescence-Associated Immune Responses in Liver Tumor Surveillance and Tumor Progression. *Cancer Cell* **30**, 533-547, doi:10.1016/j.ccell.2016.09.003 (2016).
5 Frescas, D. *et al.* Senescent cells expose and secrete an oxidized form of membrane-bound vimentin as revealed by a natural polyreactive antibody. *Proc Natl Acad Sci U S A* **114**, E1668-E1677, doi:10.1073/pnas.1614661114 (2017).