IMMUNOTHERAPY

Driving CARs to clear senescent cells

> the uPAR CAR T cells substantially decreased senescent lung tumour cells and extended survival of the mice

The accumulation of senescent cells promotes inflammation and chronic tissue damage, thereby contributing to several ageing-associated diseases. Writing in *Nature*, Lowe, Sadelain and colleagues now report the development of chimeric antigen receptor (CAR) T cells that can selectively and safely target and eliminate senescent cells in mouse disease models, reversing pathology and extending survival.

Previous studies have demonstrated that eliminating senescent cells from damaged tissues reduces symptoms and increases lifespan in mouse models of ageing-associated diseases such as cancer, fibrosis, atherosclerosis and osteoarthritis. Accordingly, there is significant interest in developing 'senolytic' agents that selectively ablate senescent cells. However, although some small-molecule inhibitors have senolytic function, most lack potency and produce major side effects.

Lowe, Sadelain and colleagues therefore focused on the alternative approach of harnessing CAR T cells directed against senescence-specific cell surface antigens.

First, to identify cell surface proteins that are specifically upregulated in senescent cells, the authors compared RNA sequencing datasets derived from three models of senescence: therapy-induced senescence in mouse lung adenocarcinoma cells, oncogene-induced senescence in mouse hepatocytes and culture-induced senescence in mouse hepatic stellate cells.

Ranking each transcript according to its magnitude of upregulation, followed by exclusion of those that were highly expressed in vital tissues, led to the identification of *PLAUR*, the gene encoding urokinase-type plasminogen activator receptor (uPAR).

Next, the authors confirmed the increased expression of uPAR on the surface of senescent cells in vitro and in vivo. In mouse lung adenocarcinoma cells and human primary melanocytes that had undergone therapy-induced and replication-induced senescence, respectively, cell surface expression of uPAR and supernatant soluble uPAR (suPAR; secreted by senescent cells) were markedly increased.

In addition, in mouse senescence-associated disease models (a patient-derived xenograft model of non-small-cell lung cancer, two different models of oncogene-induced senescence and a mouse model of carbon tetrachloride (CCl_4)-induced liver fibrosis), the number of uPAR-positive cells and the level of serum suPAR were increased.

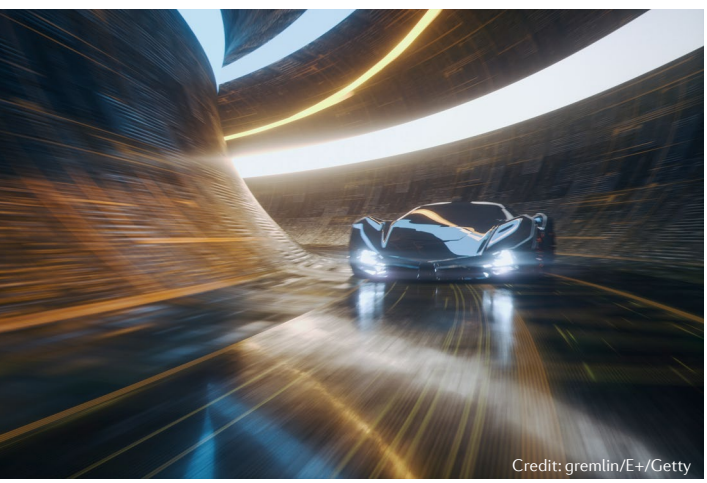
Furthermore, uPAR was demonstrated to be highly expressed in tissues from patients with senescence-associated disorders, including specimens of liver fibrosis, atherosclerotic plaques from human carotid endarterectomy specimens and in pancreatic intraepithelial neoplasia lesions from patients with pancreatic cancer.

Next, the authors constructed a uPAR-specific CAR comprising an anti-mouse uPAR single-chain variable fragment linked to human CD28 costimulatory and CD3ζ signalling domains, and transduced human T cells. The resulting uPAR-specific CAR T cells selectively and efficiently eliminated uPAR-expressing senescent cells in vitro and in vivo.

To evaluate the therapeutic efficacy of senolytic CAR T cells, the authors next transduced mouse T cells with a fully mouse uPAR CAR. When injected into a mouse model of lung adenocarcinoma treated with a senescence-inducing cancer therapy, the uPAR CAR T cells substantially decreased senescent lung tumour cells and extended survival of the mice, without signs of toxicity.

The uPAR CAR T cells were also effective in CCl_4-induced and nonalcoholic steatohepatitis-induced liver fibrosis models in mice, markedly reducing fibrosis and the number of senescent cells in the liver, in conjunction with improved liver function and reduced serum levels of suPAR in the CCl_4 model. Importantly, there was no detectable toxicity at the lower effective dose.

Together, these findings establish the broad therapeutic potential of senolytic CAR T cells for senescence-associated diseases. Further studies will be needed to determine whether uPAR-targeting CAR T cells have the required safety profile for clinical development.



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