Drug Development

Major Finding: Modified PD-1 incorporating an unnatural amino acid covalently bound PD-L1 to shrink tumors *in vivo*.

Concept: Covalent protein drugs combine advantages of small-molecule covalent drugs with proteins' selectivity.

Impact: This proof-of-concept study shows that this novel approach can be used to design new therapeutics.

NEW METHOD ENABLES DESIGN OF PROTEIN DRUGS THAT COVALENTLY BIND TARGETS

Small-molecule drugs that bind their targets covalently sometimes have advantages over those that bind noncovalently, such as increased duration of action and complete target inactivation, but a common limitation of small-molecule covalent drugs is off-target reactivity. Some protein-based drugs could hypothetically overcome these off-target effects, but proteins generally do not bind their targets covalently. Li, Chen, Klauser, and colleagues thus sought to develop a means to combine the benefits of drugs that covalently bind their targets with the advantages of protein drugs. As a proof of concept, they focused on developing a protein drug that would covalently bind PD-L1, a protein expressed on cancer cell surfaces. To do this, an unnatural amino acid, fluorosulfate-L-tyrosine (FSY), was incorporated into PD-1, the canonical receptor for PD-L1, using genetic-code expansion, with the idea being that the FSY in the modified PD-1 would react with a proximal histidine residue in PD-L1 upon binding. The modified PD-1 was able to selectively and covalently bind PD-L1 *in vitro* and *in vivo* via the intended mechanism. *In vitro*, the modified PD-1 increased T-cell activation by dendritic cells, whereas wild-type (WT) PD-1 did not. Further, in mice with humanized immune systems, the modified PD-1 exhibited potent antitumor activity; again, WT PD-1 did not produce this effect. Notably, the modified PD-1 performed equivalently to or better than the mainstay immunotherapeutic agent anti–PD-L1. Additionally, the modified PD-1, but not WT PD-1, activated chimeric antigen receptor (CAR) T cells *in vitro* and increased the CAR T cells' antitumor activity *in vivo*, promoting enhanced tumor infiltration by the CAR T cells. Together, these results not only demonstrate that the modified PD-1 examined in this work may be worth further study, but also show that this novel method to design covalent protein drugs—dubbed proximity-enabled reactive therapeutics (PERx)—is a promising approach for developing new therapies. ■

Li Q, Chen Q, Klauser PC, M Li, Zheng F, Wang N, et al. Developing covalent protein drugs via proximity-enabled reactive therapeutics. Cell 2020;182:85–97.e16.

Senescence

Major Finding: In mice, senescence-targeted CAR T cells extended survival in lung cancer and improved liver fibrosis.

Concept: Senescence is associated with pathogenesis of several diseases, such as cancer and liver fibrosis.

Impact: Targeting senescent cells using CAR T cells could be a useful strategy in cancer and other diseases.

SENOLYTIC CAR T CELLS TARGETING uPAR TREAT LUNG CANCER IN MICE

Pathologic accumulation of senescent cells is associated with inflammation that can lead to disease states such as liver fibrosis, cancer, and many others. Several small-molecule inhibitors that preferentially target senescent cells have been identified as senolytic agents, but issues including low potency and high side-effect potential have limited their utility. Amor, Feucht, Leibold, and colleagues investigated whether chimeric antigen receptor (CAR) T cells directed at the cell-surface and secreted protein urokinase-type plasminogen activator receptor (uPAR), which they found to be highly expressed by senescent cells *in vitro* and *in vivo*, could act as selective senolytics and overcome the barriers associated with previously explored small-molecule drugs. uPAR-directed CAR T cells exhibited selective cytotoxicity toward uPAR-expressing cancer cells *in vitro* and were capable of clearing hepatocytes with oncogene-induced senescent phenotypes in immunodeficient mice without showing evidence of T-cell exhaustion 15 days following administration. In immunocompetent mouse models of lung adenocarcinoma and carbon tetrachloride– or diet-induced liver fibrosis, treatment with uPAR-directed CAR T cells extended survival or reduced established fibrosis, respectively. Although toxicity was not noted in the mice treated in this study at therapeutic doses, future work will be necessary to determine whether uPAR-directed CAR T-cell treatment has an acceptable safety profile in patients. In summary, this work provides promising preliminary evidence that uPAR-directed CAR T cells effectively target senescent cells and shows that this CAR T-cell treatment has a measurable impact on disease states in immunocompetent hosts. Notably, accumulation of senescent cells contributes to the pathogenesis of several diseases not examined in this study, such as osteoarthritis, diabetes, and atherosclerosis, suggesting that testing uPAR-directed CAR T cells in models of these diseases may be of interest as well. ■

Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, et al. Senolytic CAR T cells reverse senescence-associated pathologies. Nature 2020;583:127–32.

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