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A Fresh Approach to Targeting Aging Cells: CAR-T Cells Enhance Senolytic Specificity

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Abstract

Senescence is a critical factor in several diseases, yet senolytic therapies targeting senescent cells remain hindered by lack of specificity. In the June issue of *Nature*, Amor et al. (2020) develop chimeric antigen receptor (CAR)-T cells targeting uPAR, a novel senescent-cell marker, to treat liver adenocarcinoma and liver fibrosis.

Senescent cells have been increasingly implicated in disease states ranging from cancer to atherosclerosis to Alzheimer's disease (Childs et al., 2017). These cells display heterogeneous characteristics depending on their tissue of origin, but all share the common features of permanent growth arrest coupled with resistance to apoptosis (Childs et al., 2017). Senescence also induces a distinct senescence-associated secretory phenotype (SASP) consisting of inflammatory molecules, such as vascular endothelial growth factor (VEGF) or interleukin (IL)-6, that are associated with chronic disease and tissue dysfunction (Childs et al., 2017). Small-molecule inhibitors of the BCL-2 family of anti-apoptotic proteins, as well as peptide drugs that modulate p53 function, have been developed as senolytic therapies that aim to ablate senescent cell populations (Chang et al., 2016; Childs et al., 2017). While promising, these senolytics remain plagued by imperfect specificity and potential toxicities against healthy tissue, as the targeted pathways are present not only in senescent cells but also in normal cells required for tissue homeostasis (Chang et al., 2016; Childs et al., 2017; Rudin et al., 2012).

In the June issue of *Nature*, Amor et al. (2020) present a therapeutic approach to senescence-driven pathologies utilizing chimeric antigen receptor (CAR)-T cells, a strategy best known for its efficacy against hematological malignancies in which specificity, toxicities, and limited long-term efficacy have similarly constrained therapeutic options (Amor et al.,

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2020). CARs are synthetic proteins consisting of an extracellular ligand-binding domain—most commonly a single-chain variable fragment (scFv)—fused via a transmembrane to intracellular T-cell activating and costimulatory domains such as CD28, 4–1BB, and CD3ζ (Hong et al., 2020). T cells expressing CAR molecules can be activated by the target cell-surface protein of choice, independent of major histocompatibility complex (MHC)-mediated antigen presentation. CAR-T cells directed against the B-cell marker CD19 have shown dramatic and exciting antitumor activity in both pediatric and adult B-cell malignancies, with multiple studies reporting complete response in >90% of patients (Park et al., 2016).

By comparing transcriptomic data from diseased vs. normal tissue in three different models of senescence—liver fibrosis, atherosclerosis, and pancreatic cancer—Amor et al. (2020) identified a unique senescence-specific cell surface marker, urokinase-type plasminogen activator receptor (uPAR), as a suitable target for CAR-T cell therapy. Consistent with the classical senescent cell phenotype, uPAR-positive cells in mice are nonproliferative (Ki67 negative) but express the SASP component IL-6. Notably, uPAR is poorly expressed in vital tissues, making it a promising candidate for disease-specific senolytics (Childs et al., 2017).

Amor et al. (2020) demonstrated that anti-uPAR CAR-T cells are active against an array of senescence-induced disease models (Figure 1). In an immunocompetent mouse setting, murine uPAR CAR-T cells were shown to clear $Kras^{G12D};p53^{-/-}$ lung adenocarcinoma cells that have entered a senescent state following combined MEK and CDK4/6 inhibition. These results augment the well-established role of CAR-T cells as a cancer therapeutic. Furthermore, uPAR-specific CAR-T cells were shown to reverse both carbon tetrachloride (CCl_4)- and non-alcoholic steatohepatitis (NASH)-induced liver fibrosis, as measured histologically and by liver function tests. Notably, uPAR CAR-T cells did not induce sustained toxicity in any of the mouse models tested. A major side effect associated with CAR-T cell therapy for cancer is cytokine release syndrome (CRS), a potentially fatal spike in serum cytokine levels (Hong et al., 2020). Amor et al. (2020) observed signs of early CRS-like toxicity at supratherapeutic doses of anti-uPAR CAR-T cells. However, the toxicity was transient, and could be reduced by either decreasing the CAR-T cell dose or administering immune-modulatory drugs such as inhibitors of receptors to IL-1 and IL-6. Drugs that reverse CRS may play a dual role by simultaneously neutralizing inflammatory signals from the SASP, although it is possible that certain SASP cytokines could paradoxically enhance senolytic CAR-T cell activity as well (Childs et al., 2017).

Given the variety of diseases in which senescence is implicated, it is possible that uPAR CAR-T cell therapy could have a wide range of therapeutic applications. To date, CAR-T cell therapy has primarily been applied in oncology settings. In addition to B-cell malignancies, solid tumors with target antigens such as mesothelin, HER2, and epidermal growth factor receptor variant three (EGFRvIII) are being evaluated in the clinic, with varying degrees of success (Hong et al., 2020). While solid tumors present unique challenges to CAR-T cell therapy such as immunosuppressive tumor microenvironments and barriers to T-cell trafficking, senolytic anti-uPAR CAR-T cells may prove to be an effective cancer therapeutic by exploiting senescence-induced vulnerabilities and clearing dormant tumor cells while avoiding on-target, off-tumor cytotoxicity. Furthermore, a recent study

suggests that therapy-induced tumor senescence is accompanied by vascular remodeling (Ruscetti et al., 2020), which may enhance T-cell infiltration.

Future variations of the senolytic approach may further benefit from recent developments in “armored” CAR-T cells. These CAR-T cells are engineered to express additional proteins, such as cytokine receptors or cytokines themselves, to enhance effector function, prevent T-cell exhaustion, and/or prolong persistence in the local microenvironment (Hong et al., 2020). Fusion receptors have also been developed to redirect extracellular binding of immunosuppressive molecules such as PD-L1 or TGF-β into intracellular T-cell activating signals (Hong et al., 2020). Borrowing these approaches, it may be possible to engineer senolytic CAR-T cells to not simply counteract but take advantage of the diverse molecules associated with SASP. In fact, CAR-T cells with enhanced response to molecules previously identified as SASP components (e.g., TGF-β) have been reported, making “armored” uPAR CAR-T cells enhanced by the SASP a plausible strategy (Childs et al., 2017; Hong et al., 2020).

Beyond cancer, CAR-T cells have been evaluated as potential treatment for autoimmune disease, HIV, and cardiac fibrosis secondary to myocardial disease (Aghajanian et al., 2019; Deeks et al., 2002; Ellebrecht et al., 2016). With the development of CAR-T cells as senolytics, Amor et al. (2020) add a novel dimension to the conditions that may be effectively addressed by the adoptive transfer of engineered T cells with programmed antigen specificity. Further identification of disease-specific antigens and creative applications of these living drugs will allow us to continue developing new, specific, and safe T-cell treatments for otherwise intractable diseases.

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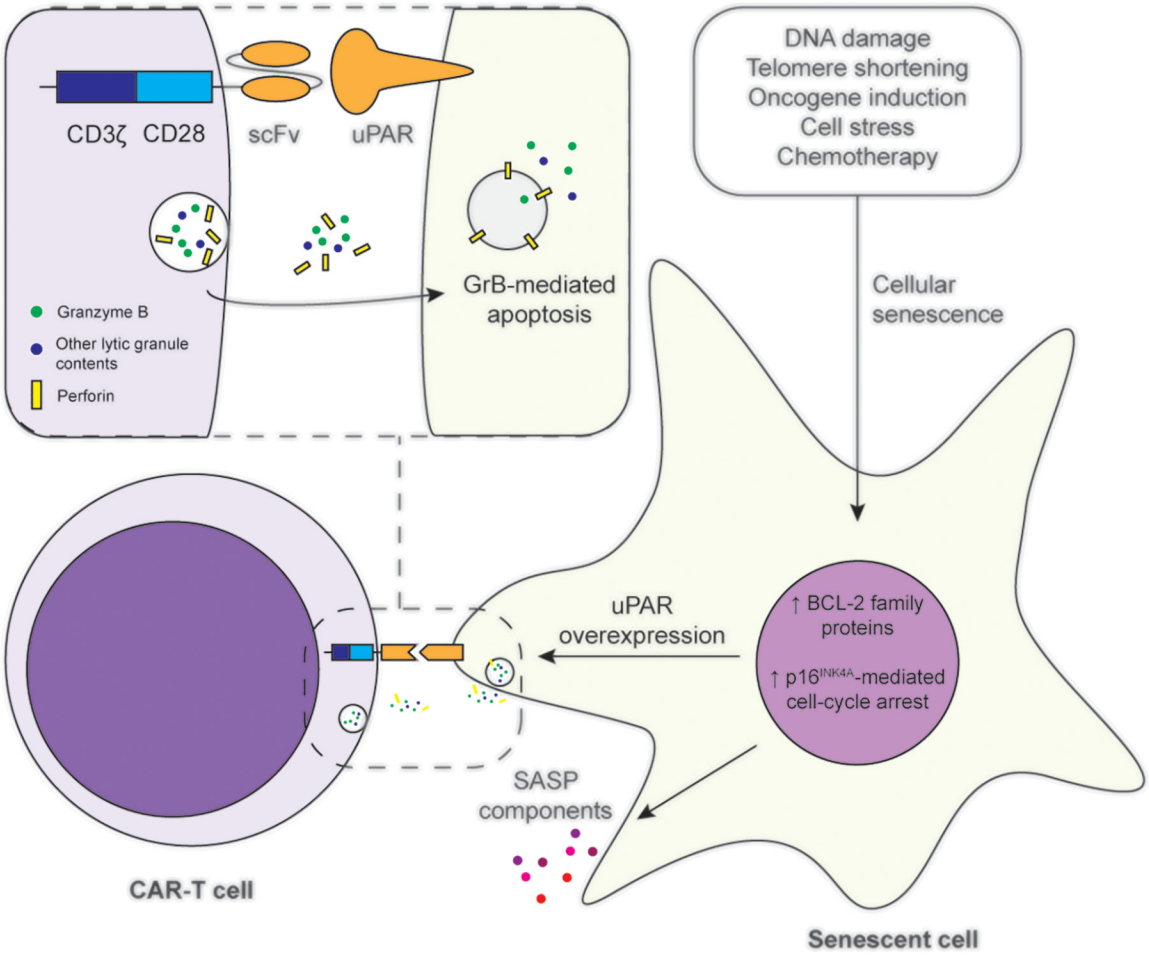

Figure 1:

uPAR CAR-T cells specifically target cells displaying a classical senescent phenotype. (Right) Various internal and external cell stressors can induce a senescent state, marked by upregulated antiapoptotic pathways, cell-cycle arrest, and expression of SASP, accompanied by overexpression of uPAR. (Inset) A CAR consisting of an extracellular uPAR-specific scFv linked to intracellular CD28 costimulatory and CD3ζ T-cell activating domains is activated by uPAR binding, resulting in T-cell activation and degranulation. Lytic granule contents released into the immunological synapse are endocytosed by the target cell, where granzyme B (GrB) and other cytotoxic molecules enter the cytoplasm via perforin channels to activate apoptotic pathways.