CARDIOVASCULAR DISEASES

Repairing cardiac injury with transient CAR-T cells

Fibrosis as a result of excessive extracellular matrix protein deposition by activated cardiac fibroblasts affects millions of patients with cardiac disease and can lead to heart failure. Unfortunately, treatment options for targeting cardiac fibrosis are limited. A study in *Science* describes a new approach to eliminate activated fibroblasts by combining the efficacy of engineered T cells with the convenience of mRNA therapeutics. Using lipid nanoparticles (LNPs) carrying mRNA encoding a chimeric antigen receptor (CAR), Rurik et al. have generated CAR-T cells in vivo that can target activated fibroblasts, attenuating cardiac fibrosis.

Cardiac fibroblasts become activated in response to myocardial injuries. In many chronic heart diseases, these fibroblasts remain pathologically activated, secreting excessive extracellular matrix, which results in fibrosis that stiffens the myocardium and impairs cardiomyocyte function. Current antifibrotic therapeutics are aimed at limiting fibrotic progression rather than at addressing fibrosis once it is established.

CARs are synthetic receptors that, when expressed on immune cells — such as T cells — isolated from the patient or healthy donors, enable them to recognize targeted antigens and trigger a specific immune response. CAR-T cell therapy has shown remarkable results in several haematological malignancies but it is also associated with important toxicity, including risks incurred by lymphodepletion prior to injection, as well high costs and complex cell manufacturing.

The authors had previously attempted to remodel established cardiac fibrosis using CAR-T cells to specifically eliminate activated fibroblasts in mice. However, indefinite persistence of antifibrotic CAR-T cells could pose a risk in the setting of future injuries, as fibroblast activation is part of the normal wound-healing process. To design a transient antifibrotic T cell, and motivated by the success of mRNA-based COVID-19 vaccines, they opted for an LNP-mRNA approach. Given that mRNA is intrinsically unstable, CAR expression on T cells would be transient, and no CAR-T cell would persist for more than a week.

"We used our established T cell-targeted LNP-mRNA platform to reprogram T cells in vivo and to avoid removing T cells from the patient," explains Hamideh Parhiz, one of the lead authors of the study. This approach would also reduce the significant cost, risk and extensive infrastructure required for ex vivo CAR-T cell generation, she adds.

First they generated modified nucleoside-containing mRNA encoding a CAR against fibroblast activation protein (FAP), a marker of activated fibroblasts. This mRNA was packaged in LNPs targeting CD5, which is naturally expressed by T cells. In vitro, CD5-targeted LNPs delivered the mRNA to most (83%) T cells in culture. CAR expression peaked at 24 hours and rapidly decreased over the following days. The newly generated CAR-T cells effectively eliminated FAP-expressing target cells in vitro in a dose-dependent manner.

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Next, the authors assessed the antifibrotic abilities of these CAR-T cells in vivo, in an established hypertensive mouse model of cardiac fibrosis induced by constant infusion of angiotensin II and phenylephrine.

A week later, once fibrosis was established, LNPs carrying the FAP CAR mRNA were injected intravenously. The authors were able to detect a consistent population of FAP CAR$^+$-T cells (17.5–24.7%) 48 hours after injection. Expression of FAP CAR lasted for approximately a week, underlining the desired transient effect of the approach. After two weeks, the authors observed significant improvements in cardiac functions in treated mice: left ventricular (LV) diastolic function returned to uninjured levels and LV systolic function was also considerably improved.

Histologically, there was a significant reduction of the overall burden of extracellular matrix. Importantly, neither B cells or NK cells expressed FAP CARs, and there were no histological changes in non-cardiac organs or weight loss, suggesting the specificity and safety of the approach.

"Generation of engineered T cells in vivo using mRNA is attractive for certain disorders because the transient nature of the produced CAR-T cells is likely to limit toxic effects and allow for precise dosing," explains Parhiz.

Jonathan Epstein, another lead author of the study, believes this example shows the potential of combining these cutting-edge approaches for treating a broad range of diseases with large unmet medical needs. "Fibrosis, for example, can affect nearly every organ and tissue, yet we have very few effective therapies. If we can decrease the burden of disease using an mRNA-based immunotherapy, we may be able to improve the quality of life for many patients in need," he says.

The authors are considering extending these studies to large animals and eventually to patients. "We are eager to do the necessary safety and efficacy studies to help to see this to happen," concludes Epstein.

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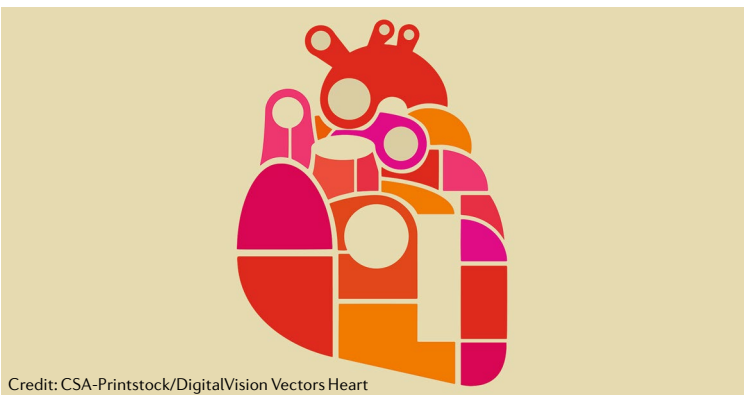

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