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Safer, more effective cell therapies enabled by coupled gene knock-in and knockout

Stanford researchers have developed a strategy for engineering next-generation cell therapies where gene knock-in is tightly coupled to gene knockout, preventing dangerous side effects associated with cells that have the knockout in the absence of the knock-in and vice versa.

The use of more sophisticated genetic modifications, like combined gene knockout/knockin, has significantly expanded the scope of cell therapies. For example, knocking out immune checkpoint proteins in CAR-T cells can endow resistance to tumor immune suppression. However, current methods for engineering cell therapies can generate cells that have the knockout in absence of the knock-in or vice versa, which can cause severe autoimmune side effects.

To address this issue, Stanford researchers developed a method for cell engineering that tightly links gene knockout to knock-in. Here, a DNA cassette is introduced that codes for the gene knock-in as well as the guide RNA for gene knockout. Unlike prior methods where the guide is expressed from a U6 promoter, the guide is instead incorporated into an mRNA. In this way, the guide can only be translated after successful knock-in. This not only means that gene knockout and knock-in are tightly linked, but also that each event is separated in time, obviating genotoxicity risks associated with multiple simultaneous DNA breaks.

Stage of Development
Proof of concept: demonstrated knockout alongside knock-in in T-cells

Applications

- Cell therapies (CAR-T, TCR-T, CAR-NK, etc.)

- Autologous and allogeneic cell therapies
- Preclinical discovery using pooled knock-in/knockout libraries

Advantages

- Enables more sophisticated, multi-step cell engineering
- Gene knockout and knock-in are tightly linked
- Obviates autoimmune risks associated with knock-in in the absence of successful knockout (and vice versa)
- Decreases risks of genotoxicity associated with simultaneous knockout and knock-in

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