SynthImmunol_NMU Notebook No.2

Time: 2024.04.07-2024.04.13

1. **Experiment:** Lecture research and report
2. **Time:** 2024.04.07-2024.04.27
3. **Member:** Song Zhang, Yaqi Gao, Xinyu Zhu, Xiaoyuan Chen, Yinran Luo, Hanyue Liu, Xudong Tang
4. **Summary:**

(1) Lecture 1: Decoding CAR T cell phenotype using combinatorial signaling motif libraries and machine learning

① Time: 2024.04.07-2024.04.13

② Member: Song Zhang, Yaqi Gao

③ Summary:

CAR T cell therapy has been approved officially or put into clinical trials are mainly anti-CD19 CAR T whose costimulatory domain is simple. Nowadays, the costimulatory domain library is limited, so it's necessary to create a new CAR T costimulatory domain library. Signaling motifs are the basic components of the costimulatory domain. With the help of machine learning, the author created predictive models to characterize the correspondence of signaling motifs to CAR T-cell phenotypes and extracted design rules to identify signal motif compositions that could increase both CAR T cell killing capacity and persistence.

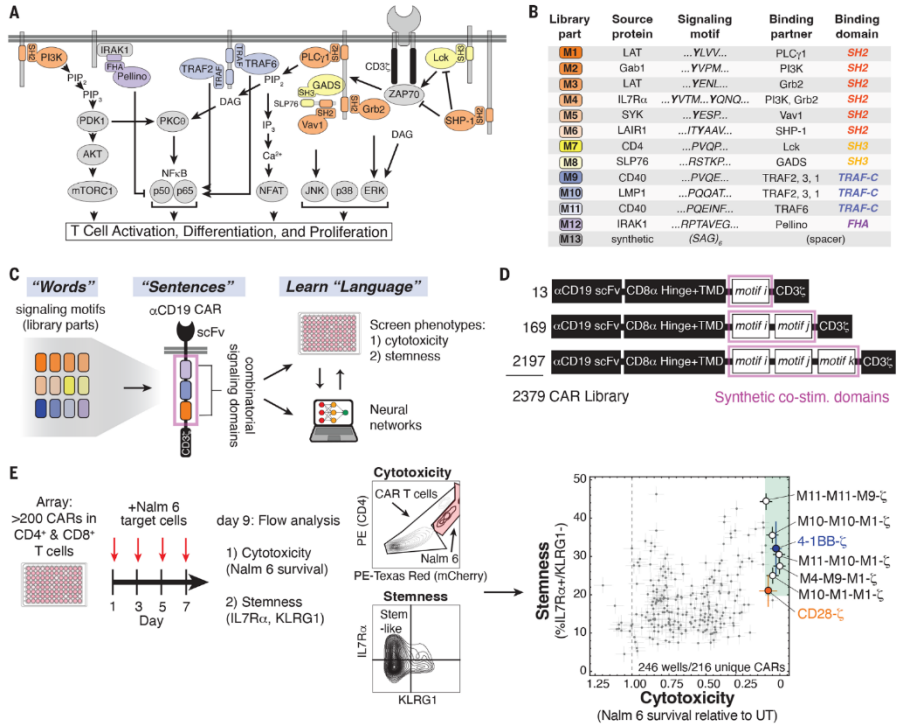

Fig. 1 CAR costimulatory domains with synthetic signaling motif combinations generate diverse cell fates with decoupled cytotoxicity and stemness.

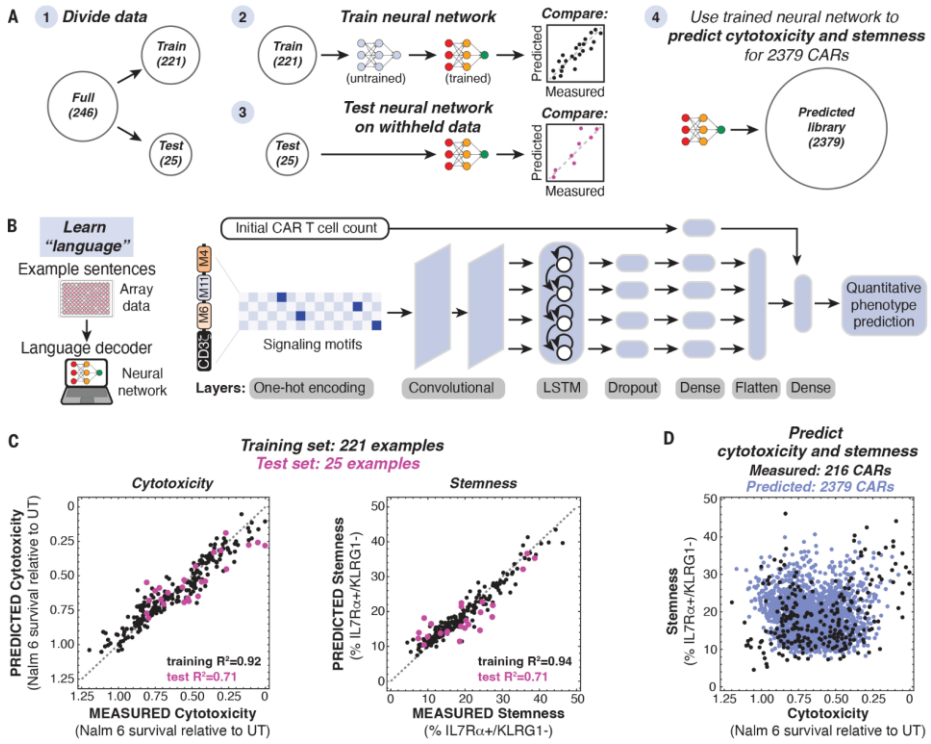

Fig.2 Neural networks decode the combinatorial language of signaling motifs to predict cytotoxicity and stemness of novel motif combinations.

(2) Lecture 2: Modular pooled discovery of synthetic knockin sequences to program durable cell therapies

① Time: 2024.04.14-2024.04.20

② Member: Xiaoyuan Chen, Hanyue Liu, Xudong Tang

③ Summary:

Chronically stimulated T cells can differentiate into a dysfunctional state characterized by epigenetic changes, decreased cell proliferation, and decreased cytokine production, and they express PD-1 and LAG-3. This T-cell dysfunction with exhaustion features has been identified as a major cause of suboptimal response to therapy.

There are three main ways in which genome engineering can enhance T-cell fitness.

First, regulation of signaling through targeted CAR integration regulated by the promoter of the endogenous TCRα chain. Second, use CRISPR-Cas9 to remove genes that limit persistent T-cell function. Third, perform gain-of-function screening with lentiviral libraries of CRISPR-activated (CRISPRa) or open reading frames (ORFs). However, these gain-of-function screening approaches have not incorporated antigen-specific TCR or CAR on a large scale in primary human T cells and CRISPRa screening could not detect synthesized gene products.

The author put forward that the state of TCR-/CAR-T cells can be engineered by directly modulating transcriptional regulators or synthetic surface receptors to alter the cellular response to external

stimulation.

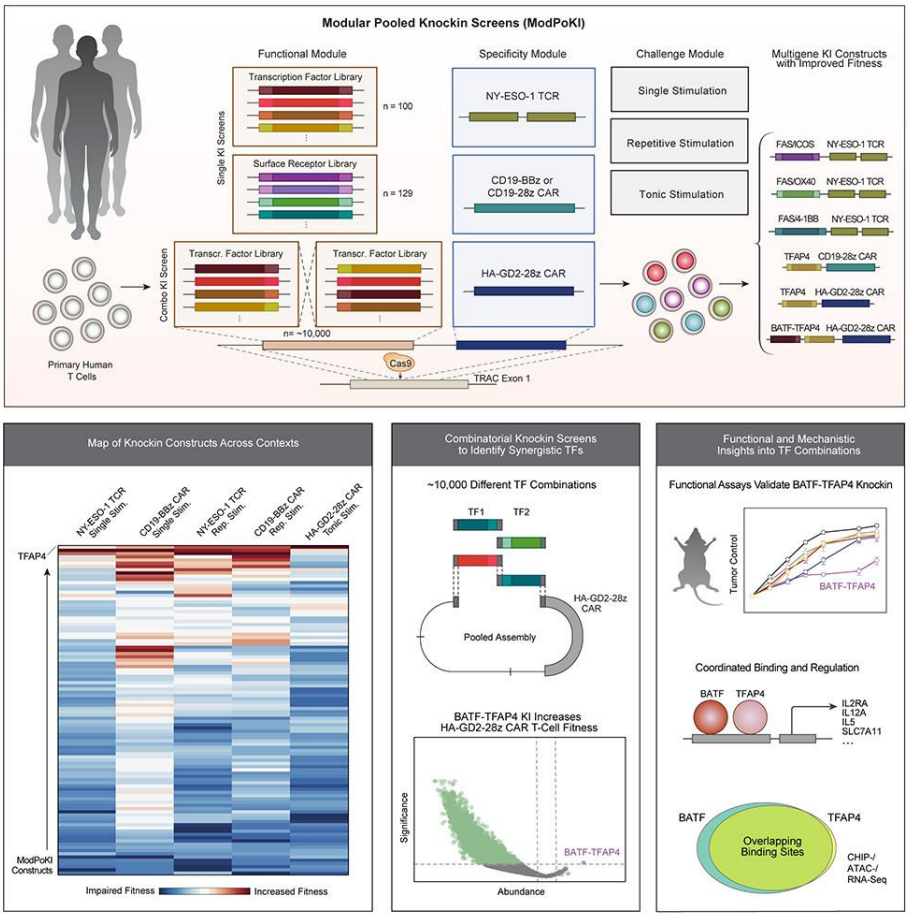

Fig.3 Modular pooled knockin screening (ModPoKI) is an adaptable platform that enables the evaluation of hundreds to thousands of different T cell constructs for engineered cellular immunotherapies.

(3) Lecture3: Modular design of synthetic receptors for programmed gene regulation in cell therapies

① Time: 2024.04.21-2024.04.27

② Member: Xinyu Zhu, Yinran Luo

③ Summary:

Synthetic biology has established powerful tools to precisely control cell function. Engineering these systems to meet clinical requirements has enormous medical implications. The author adopted a clinically driven design process to build receptors for the autonomous control of therapeutic cells. They examined the function of key domains involved in regulated intramembrane proteolysis and showed that systematic modular engineering can generate a class of receptors that they call synthetic intramembrane proteolysis receptors (SNIPRs) that have tunable sensing and transcriptional response abilities. The author demonstrates the therapeutic potential of the receptor platform by engineering human primary T cells for multi-antigen recognition and production of dosed, bioactive payloads relevant to the treatment of disease. The design framework enables the development of fully humanized and customizable transcriptional receptors for the programming of therapeutic cells suitable for clinical translation.

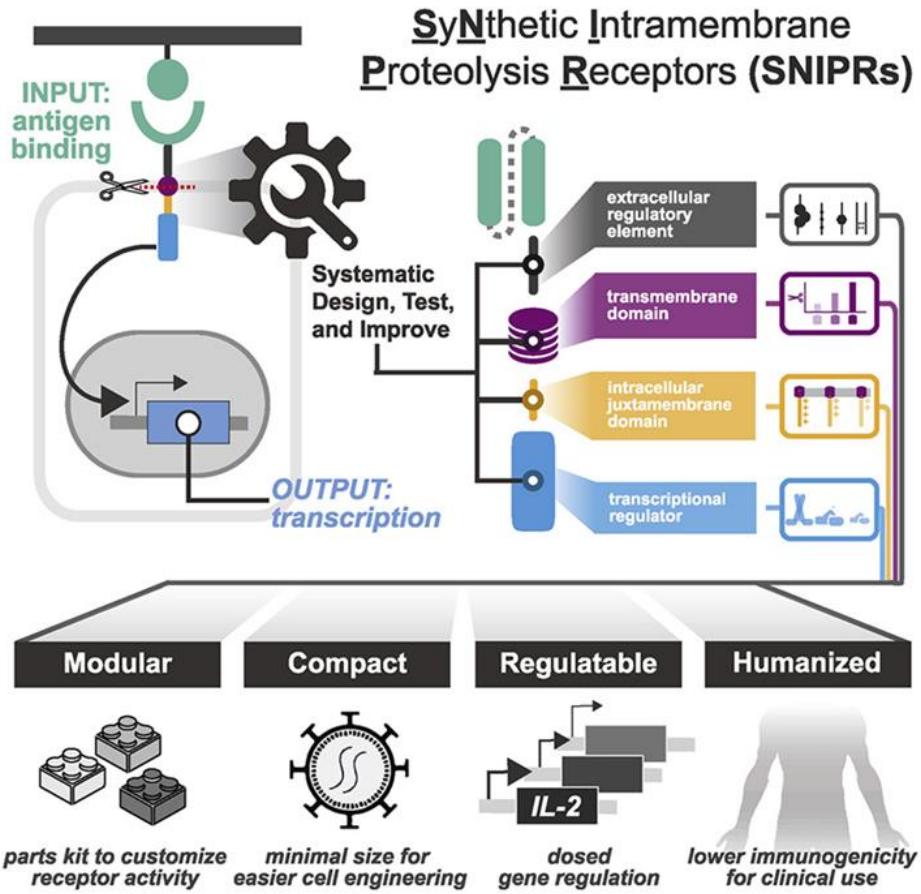

Fig.4 The design framework for fully humanized transcriptional receptors for the programming of therapeutic cells.