High-throughput tools for decoding T cell receptor specificity

By
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T cells are central to adaptive immunity by recognizing specific antigens through T cell receptors (TCRs). These receptors bind to peptides presented by major histocompatibility complex (pMHC) proteins, driving targeted immune responses in cancer, infection, and autoimmunity. Decoding how TCRs recognize pMHC antigens is crucial to achieving the full potential of cancer immunotherapies and to identifying therapeutic targets in autoimmunity, infectious disease, and allergy. Despite the clinical importance of decoding TCR-antigen recognition, existing experimental methods are highly constrained by scale and cost and have explored a negligible fraction of possible TCR-antigen pairs. This lack of data is a substantial barrier to computational approaches predicting TCR specificity. High-throughput, accessible methods to screen TCRs and antigens at scale remain a critical unmet need.

This thesis presents two advances enabling large-scale TCR-antigen screening. First, we address the major cost, labor, and scale challenges of assembling and screening TCRs from sequences. We present a scalable and cost-effective platform for pooled synthesis and screening of synthetic TCR libraries. Our modular assembly strategy enables the construction of tens of thousands of TCRs for under $1 per TCR, a 100-fold decrease in cost, with >99% accuracy. We integrate this approach with RAPTR, a high-throughput antigen discovery platform, to enable screening of thousands of TCRs against hundreds of antigens simultaneously. Using this system, we screen 3,808 TCRs from donors with vitiligo and 30,810 TCRs from pancreatic ductal adenocarcinoma tumor tissue to identify antigen-reactive TCRs. This broadly accessible pipeline reduces cost and technical barriers to large-scale TCR screening, enabling unbiased antigen reactivity profiling and expanding the known landscape of TCR-antigen interactions. We anticipate widespread adoption of this TCR assembly approach and its seamless integration with other antigen discovery workflows.

The second aim introduces a high-throughput antigen discovery approach integrating yeast display with pMHC-displaying viruses. Yeast display is well-precedented for screening libraries of $>10^8$ members against single pMHCs or TCRs. Despite its scale, ease of use, and robustness, yeast display is constrained by the need to screen against laboriously produced individual proteins. We explore the compatibility of pMHC-displaying virus-like particles (VLPs) as a simple means of screening yeast display libraries. Using libraries of VLPs, we screen millions of yeast-displayed TCR variants against libraries of antigens to capture subtle differences in cross-reactivity with mutations to the highly variable CDR3 regions of TCRs. We envision this approach as a means of rigorously testing candidate therapeutic TCRs and generating large training datasets for deep learning models of TCR recognition. By overcoming key technical barriers, these tools significantly expand our ability to study TCR specificity and engineer new antigen-specific therapeutics.

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