Integrated, single-cell analysis of transcriptional phenotype and clonotypic identity

by

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Abstract

Single-cell RNA sequencing (scRNA-seq) currently affords the ability to resolve whole transcriptomes of single cells with substantial throughput and has revolutionized studies of gene expression. Recent technical advances have enabled the matching of T cell receptor (TCR) and B cell receptor (BCR) variable region sequences to the transcriptional profiles of the same cells, but methods to perform analysis of the resulting data, which would enable the analysis of antigen-specific T and B cell phenotypes in their clonotypic context, remain limited. In this thesis, we develop strategies to integrate the analysis of single-cell transcriptional states with the analysis of TCR and BCR clonotypes. We demonstrate how these strategies can be used to develop actionable biological insights across a diverse spectrum of immunological contexts.

In the first part of this thesis, we profile CD8+ T cells recovered from murine tumors established in either the flank or the lung. Using scRNA-seq, we observe that common tumor-reactive clonotypes in these tumors exhibit phenotypic skewing dependent on the tissue site of tumor growth. We demonstrate that this phenotypic skewing is established during T cell priming in either the mediastinal or inguinal lymph node and results in a lack of responsiveness to immune checkpoint blockade (ICB) therapy in lung tumors. We show that gene expression signatures associated with this phenotype are present in sequencing data generated from patients with non-small cell lung cancer, suggesting that inadequate T cell priming may contribute to ICB resistance in human patients.

In the second part of this thesis, we analyze T cells recovered from the esophageal biopsies, duodenal biopsies, and peripheral blood of patients with the allergic disease eosinophilic esophagitis (EoE) with scRNA-seq. We identify a clonally expanded, pathogenic effector Th2 (peTh2) cell phenotype that is associated with EoE. This phenotype demonstrates features of an antigen-specific T cell response, including convergence of TCR sequences. It also exhibits an association with the homing marker GPR15, which is upregulated among peTh2 clonotypes in the peripheral blood that were simultaneously detected in esophagus. Additional investigations further support that

peTh2 cells in EoE likely possess specificity for food allergen-derived epitopes and exhibit enhanced esophageal homing potential associated with expression of GPR15.

In the last part of this thesis, we develop a methodology to enable the recovery of paired, full-length BCR sequences from 3`-barcoded scRNA-seq libraries. The method is simple, cost-efficient, and can be applied retrospectively to archived samples. We first establish the accuracy of this method. We then apply it to reveal clonal relationships both among duodenal plasma cells in patients undergoing diagnostic screening for EoE and antigen-specific B cells elicited by vaccination in rhesus macaques.

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