10x GENOMICS

Research Snapshot:

Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment

Researchers at Memorial Sloan Kettering Cancer Center (New York, NY, USA) performed large-scale, single cell analysis of immune cells within the breast tumor microenvironment. They developed computational tools to normalize and cluster single cell RNA sequencing data across multiple tumors. This revealed a continuum of activation and differentiation states for tumor-associated T cells and macrophages.[1]

Snapshot

RESEARCH AREA	Tumor microenvironment
ORGANISM	Homo Sapiens
SAMPLE TYPE	Human breast carcinomas and matched normal controls
RESEARCH QUESTIONS	How do phenotypes of immune cells within tumors differ compared to normal tissue? How does the tumor microenvironment shape the phenotypes of immune cells?

10x Product

Chromium Single Cell Immune Profiling

- Chromium Single Cell 5' Library and Gel Bead Kit
- Chromium Chip A Single Cell Kit
- Chromium i7 Multiplex Kit
- Chromium V(D)J Enrichment Kit, Human T Cell
- Chromium Single Cell 5' Library Construction Kit

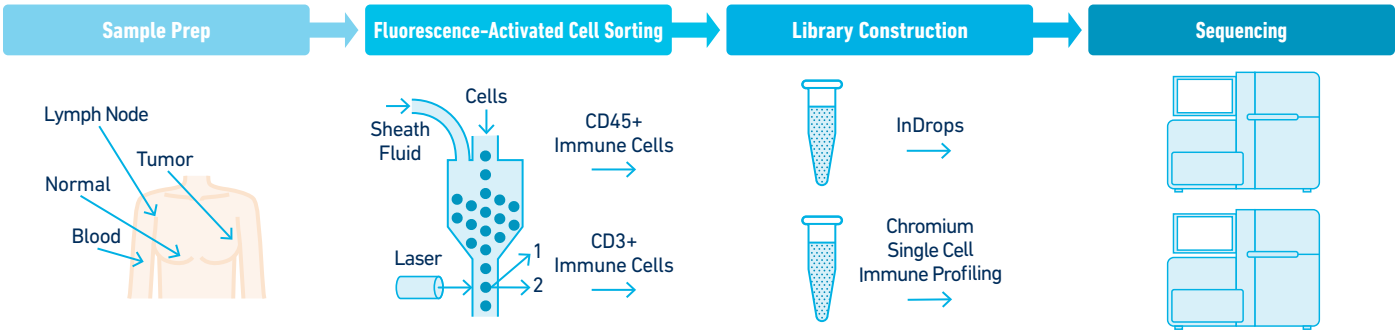
Experiment Overview

Single cell transcriptional profiling of 45,000 breast carcinoma-resident immune cells (1)

- Tumor tissue from 8 treatment-naïve breast carcinoma patients, including ER+, PR+, HER2+, and triple-negative tumor subtypes
- Matched normal breast tissue, blood, and/or lymph node samples from 4 of the patients
- Fluorescence-activated cell sorting to enrich for CD45+ immune cells
- Single cell RNA-seq using a custom-built InDrop microfluidics platform
- Libraries were sequenced on an Illumina HiSeq 2500 instrument

Single cell immune profiling of 27,000 breast carcinoma-resident T cells (2)

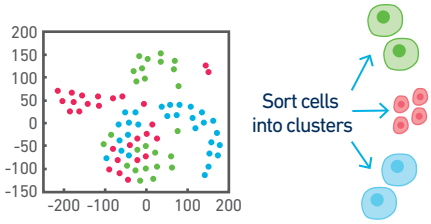
- Tumor tissue from 3 additional treatment-naïve breast carcinoma patients
- Fluorescence-activated cell sorting to enrich for CD3+ T cells
- Single cell RNA-seq plus full-length, paired T-cell receptor (TCR) sequencing using the Chromium Single Cell Immune Profiling Solution from 10x Genomics
- Gene expression libraries were sequenced on an Illumina NextSeq instrument; V(D)J-enriched libraries were sequenced on an Illumina HiSeq 2500 instrument



Computational Analysis

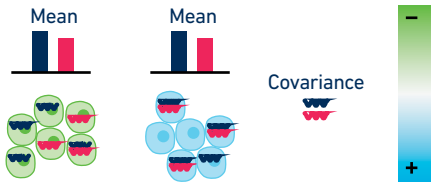
BISCUIT

To systematically compare single cell data across multiple tumor micro-environments, the authors developed BISCUIT (Bayesian Inference for Single-cell ClUstering and ImpuTing.)[2] BISCUIT robustly identified distinct immune cell populations using both mean gene expression and covariance patterns



Covariance

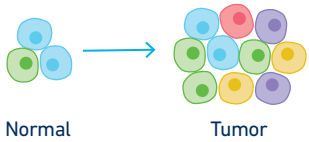
Covariance is a feature that demonstrates the power of single cell data. As illustrated here, these two cell populations look the same if measured in bulk by average gene expression profiles. However, single cell RNA-seq unmasks the true heterogeneity.



Results

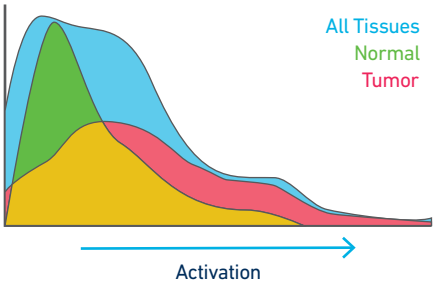
Expansion of immune cell phenotypes within tumors

Single cell transcriptional profiling helped determine an atlas of immune cell phenotypes in the breast cancer microenvironment. Researchers observed phenotypic overlap between normal and tumor-tissue resident immune cells, but also significant expansion of total variation among cells within tumors. Rather than rapid transition to a few, discrete differentiation states, diversity of immune cell activation occurred along a continuum.



Continuous T cell activation

Chromium Single Cell Immune Profiling data allowed direct mapping of gene expression to TCR utilization by the same individual cells to reveal the activation states of individual T cell clones. This analysis indicated that TCR diversity only partially accounts for the continuous spectrum of T cell phenotypes. Detailed immune profiling data demonstrated that T cell phenotypes are likely shaped by antigen distribution across the tumor microenvironment.



References

[1] Azizi, Carr, Plitas, Cornish, Konopacki et al., Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment. Cell. 5, 1293-1308 (2018)

[2] BISCUIT: omictools.com/biscuit-tool

For a full list of publications visit **10xgenomics.com/publications**

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