Immunoinformatics
-
Computational approaches to study the human immune system

Katharina Imkeller
University Hospital Frankfurt and Frankfurt Cancer Institute
CSAMA 2022
Goals for this lecture

Get to know computational approaches to study:

1. Cell types and phenotypes
2. Interactions of immune cells
3. Antigen specificity
1. **Cell types and phenotypes**
2. Interactions of immune cells
3. Antigen specificity
How immunologists usually define cell types

FACS gating for cell type identification
Identification of cell types by clusters of differentiation (CD)

371 defined clusters of differentiation

Historical origin: grouping of antibodies that bind to the same cell surface antigen.

Challenges for immunoinformatics:
- consistent usage of CD nomenclature dependent on field of immunology
- CD nomenclature does not always correspond to protein name/gene name
- antibody binding != surface marker expression != gene expression
Cell type assignment in the single cell transcriptomics analysis workflow

Very likely:
scRNA cell type not exactly identical to FACS cell type

scSeq analysis workflow
(see single-cell lecture by D. Risso)

Amezquita et al 2022
Manual annotation using marker gene detection

*Bioconductor package: scran*
*Functions: scoreMarkers(), findMarkers()*

**T-SNE of cells coloured by cluster**

**Expression of marker genes**

Dataset: 10x PBMC example data
Automated cell type annotation using Bioconductor

*Bioconductor packages: SingleR, celldex*

```r
ref <- BlueprintEncodeData()
pred <- SingleR(test = sce, ref = ref, labels = ref$label.main)
```

---

**Step 1:** Identifying variable genes among cell types in the reference set

**Step 2:** Correlating each single-cell transcriptome with each sample in the reference set

**Step 3:** Iterative fine-tuning—reducing the reference set to only top cell types

Aran et al. *Nature Immunology* (2019)
SingleR returns prediction scores and cell type labels

Scores for cell type assignment
Scores for assigned labels are indicated in red

Result of automated cell type assignment

Reference: BlueprintEncodeData()
Dependent on the reference, the predictions may change

HumanPrimaryCellAtlasData()

BlueprintEncodeData()

MonacoImmuneData()
sc-seq datasets generated using different experimental method

**10x genomics 3**
Whole transcriptome, poly(A)-enrichment

**BD Rhapsody targeted**
Panel sequencing (primers for ~ 4000 genes)
Whole transcriptome reference to annotate a targeted sequencing dataset

**Manual annotation**

- CD4+ CD25+ FOXP3+ Tregs
- CD4+ CD26+ CD45RO+ KLRB1+ memory
- CD4+ CD5+ CD6+ cytotoxic
- CD4+ naive
- CD4+ NK-like FCGR3A+
- CD4+ RGS1+ ICOS+ memory
- CD8+ CD45RA+ FCGR3A+ effector memory
- CD8+ effector memory
- CD8+ naive
- CD8+ tissue resident memory
- CD8+ TNF+ IFNG+ effector memory
- gamma/delta T cell
- NKT cells

**SingleR annotation**

- B cells
- Basophils
- CD4+ T cells
- CD8+ T cells
- Dendritic cells
- Monocytes
- Neutrophils
- NK cells
- Progenitors
- T cells
Take home messages for cell type assignment

Automated cell type assignment:

- Works well for common cell populations sequenced with whole transcriptome sequencing.
- Does not work well if you enrich for rare cell populations (NKT cells, atypical B cells)
- Does not work well for other sequencing approaches.

Recommendations from my own experience:

- Check that the markers you expect are also expressed in the clusters.
- If you have many different cell types, split the data into subpopulations (B cells, T cells, tumor cells…). Independent subsequent analysis.
- After annotating, save an annotated intermediate object for downstream analysis.
Outline of the lecture

1. Cell types and phenotypes
2. **Interactions of immune cells**
3. Antigen specificity
Ligand - Receptor interactions

Here: Receptors and ligands encoded in the germline

Experimental measurements:
- Mass spectrometry of complexes
- Binding assays
- Affinity measurements

Databases of ligands and receptors:
- Cellinker
- CellChat
- CellPhoneDB
- iCELLNET
- ...

Armingol et al. Nat Rev Genetics (2021)
Workflow for scoring receptor-ligand interactions

1. Collection of samples or cells for transcriptomic analysis
2. Data preprocessing and generation of expression matrix
3. Interacting proteins or ligand-receptor pairs
4. Filtering by interacting proteins or ligand-receptor pairs
5. Cell-cell interactions and communication analysis
6. Interpretation and visualization of results

Armingol et al. Nat Rev Genetics (2021)
Different scoring methods

Please see reference for complete list of tools and methods…

Armingol et al. Nat Rev Genetics (2021)
Application: Identifying receptor-ligand interactions between cell types

Vento-Tormo (2018)
Outline of the lecture

1. Cell types and phenotypes
2. Interactions of immune cells
3. Antigen specificity
Adaptive immune receptors are specific for a particular antigen
Annotating immunoglobulin sequences

<table>
<thead>
<tr>
<th>Features</th>
<th>V,D,J usage</th>
<th>CDR/FWR</th>
<th>Somatic hypermutations</th>
</tr>
</thead>
</table>

Annotation tools: IgBLAST, Immcantation, …
AIRR exchange format

Standard format for annotating adaptive immune receptor sequences

AIRR common repositories

Archives for AIRR sequences and metadata e.g. iReceptor public archive
Adaptive immune receptor repertoire (AIRR)

**Repertoire:**

All B or T cells with their antigen receptor present in an individual at a given time

- $\sim 10^{12}$ possible combinations
- “Public” receptors are extremely rare

*Imkeller, Wardemann (2018)*
Single-cell sequencing including AIRR (VDJ-seq)

First single-cell receptors sequenced long before the single-cell era!

Challenge: maintain heavy/light chain association.

Wardemann, Busse (2017)
10x genomics VDJ sequencing: Linking heavy and light chain via the cellular barcode
10x genomics VDJ - read annotation
TCR Clonotypes and single cell transcriptomic data

Summary

Immune cell types and interactions

- How to automatically annotation immune cell types (or not).
- How to score receptor ligand-receptor interactions in expression datasets.

Antigen specificity and adaptive immune receptor repertoires

- How to annotate adaptive immune receptor repertoires (bulk and single-cell).
Further reading

OSCAR: https://bioconductor.org/books/release/OSCA/


Review cellular interactions:


Review B/T cell repertoires:
