Single Cell Sequencing

SFRP2/DPP4 and FMO1/LSP1 Define Major Fibroblast Populations in Human Skin
Tracy Tabib, Christina Morse, Ting Wang, Wei Chen and Robert Lafyatis

Vera Vorstandlechner
22.10.2018
RNAseq

- "Transcriptome" = total amount of all mRNA present in a cell, = all genes transcribed at the moment
- cDNA = DNA processed from RNA using reverse transcriptase
- RNAseq = sequencing of the transcriptome from cDNA
- ~15,000 genes per sample
High throughput sequencing

- „Next Generation Sequencing“
- Massively parallel sequencing
- ChIP-Seq
- Sequencing by synthesis (Illumina)
- ...

Wikipedia.org/DNA-sequencing
Single cell sequencing

(i) sequencing adapters and primers
(ii) 14 bp barcode
(iii) 10 bp randomer to index molecules (unique molecular identifier, UMI)
(iv) an anchored 30 bp oligo-dT to prime polyadenylated RNA transcripts

Zeng GXY et al. 2017
Barcoded Single Cell Gel Gel Beads

Cell suspension → Sorting + Barcoding → Barcoded cDNA

→ max. 10,000/sample → ~15,000 genes/sample

10xgenomics.com
Data processing

- 10x Genomics: CellRanger pipelines
  - CellRanger mqfast: demultiplexing of raw data
  - CellRanger count: alignment, filtering, barcode counting, and UMI counting, generate gene-barcode matrices, determine clusters, and perform gene expression analysis
  - CellRanger aggregate: aggregates outputs from several samples
Data processing

- Secondary analysis: R-package „Seurat“, Loupe Cell Browser
Cells of the skin

**Epidermis**
- Keratinocytes
- Langerhans-Cells
- Melanocytes
- Merkel-Cells

**Dermis**
- Fibroblasts
- Endothelial cells
- Mast cells, granulocytes, monocytes, etc.

https://pl.wikipedia.org/wiki/Plik:Skin_layers.png
Figure 1
Tabib et al 2018
Feature plots of cluster markers

- **DES**: Smooth muscle cells
- **KRT1**: Keratinocytes
- **SCGB1B2P**: Secretory/glandular cells
- **CD3D**: T-cells
- **RGS5**: Pericytes
- **KRT14**: Keratinocytes
- **LOR**: Cornified envelope
- **AIF1**: Dendritic cells
- **VWF**: Endothelial cells
- **PMEL**: Melanocytes
- **COL1A1**: Fibroblasts
- **IGJ**: B-Cells

*Figure 2, Tabib et al. 2018*
Hierarchical Clustering of fibroblasts

Figure 3
Tabib et al 2018
Re-Running Clustering for fibroblasts only

Fibroblasts = cells expressing Col1A1, Col1A2 & PDGFRA

Supplementary Material
Tabib et al 2018
Gene expression in fibroblast subpopulations

Supplementary Material
Tabib et al 2018
Gene expression in fibroblast subpopulations

Supplementary Material
Tabib et al 2018
IF staining in normal skin showing fibroblast subpopulations

Figure 4
Tabib et al 2018
### Differentially expressed genes per cluster

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Significant genes female vs. male

Supplementary Material
Tabib et al 2018
Kang HM et al. 2018

Tutorial: Stimulated and unstimulated PBMCs:
https://satijalab.org/seurat/immune_alignment.html
Kang HM et al 2018

Tutorial:
Stimulated and unstimulated PBMCs:
https://satijalab.org/seurat/immune_alignment.html
“Single-Cell RNA-Seq Analysis Maps Development of Human Germline Cells and Gonadal Niche Interactions”
“Tracing the temporal-spatial transcriptome landscapes of the human fetal digestive tract using single-cell RNA-sequencing”
Gao S. et al 2018
“Prospectively Isolated Tetraspanin+ Neoblasts Are Adult Pluripotent Stem Cells Underlying Planaria Regeneration”
Tabula muris: Single cell transcriptomics from 20 mouse organs

Tabula muris consortium, 2018
Conclusion

• New perspectives for high-resolution genetic analyses

• Applications:
  • Single cell genomics (e.g. microbiome)
  • Single cell transcriptomics: gene expression, immune profiling,
  • Single cell epigenetics
  • Linked-reads genomics: whole genome-sequencing, exome sequencing,
    de novo assembly

• Complex bioinformatic process and data visualization

• For developmental studies, substance-effect studies, microbiome
  screening
Discussion

• Pros/Cons?
• Applicability
• Applications
• Future directions
• …

Questions? Thank you!
References