SCENE: A Framework for Single-cell Gene Co-expression Network Analysis Across Cell Types and Conditions

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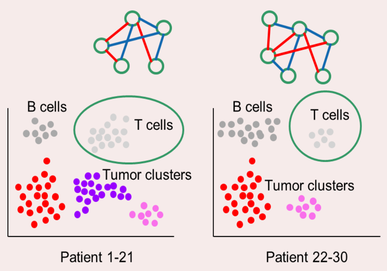
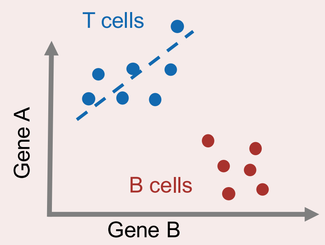
1 Introduction

Gene co-expression networks
are governing all cellular processes in health and disease. They are likely context specific (e.g. celltype, tissue, disease, patient)



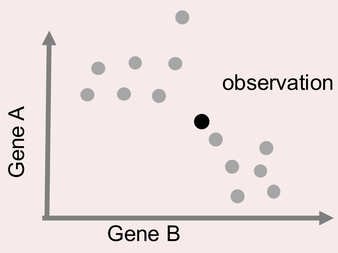
Single cell samples

- Snapshot of co-regulated expression
- Co-expression within cell types



Bulk samples

- $\mathrm{Cor}(\bar{x}, \bar{y}) \neq \overline{\mathrm{Cor}(x, y)}$
- Averaging across cell types
- Compositional effects



Aim

Construction of cell type-specific gene co-expression networks to discover context-dependent re-wiring of biological pathways.

3 Dataset

CIMA (Chinese Immune Multi-Omics Atlas)[4]

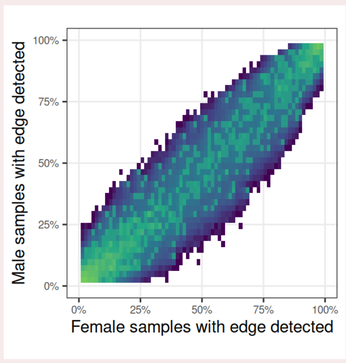
- Population: 421 healthy individuals, 20-77 years old, 56% female.
- scRNA-seq from PBMC (peripheral blood mononuclear cells) - circulating immune cells.
- 6'484'974 cells total (15'478 cells per individual)

Plasmacytoid dendritic cells (pDC)

- Rare innate immune cells, respond to viral nucleic acids (via TLR7/9), professional IFN-I producers
- Implicated in autoimmune diseases, i.e. systemic lupus erythematosus and psoriasis
- Female pDCs respond more intensely to viral infection

Comparison: pDC - sex

- 70 female and 41 male samples (111 samples together, which pass min cell number threshold)
- > 3M edges for ~2500 PID genes were identified. 67.5% of edges passed the min sample number thresholds.
- 6.0% of edges were Female-only and 1.6% Male-only.
- Edge fraction distribution correlates at 0.99 in Female and Male.



2 SCENE workflow

Comparison
Choose based on research question

Cell type + metadata covariate (e.g. group variable)

OR

cell type 1 vs cell type 2

Comparison must have ≥ 20 samples (≥5 per group) with ≥100 cells per cell type

1 Data prep
- Normalization
- Log-transformation
- Filtering

Single cell gene expression count table

2 Edge weights

Topological overlap measure (TOM)[1]

Sample*-level edge** weights

3 Normalization
- Transformation: $\log\left(\frac{weight}{1-weight}\right)$
- Quantile normalization

4 Statistical testing

Moderated t-test with `limma`[2]

Edge statistics for the comparison chosen for analysis

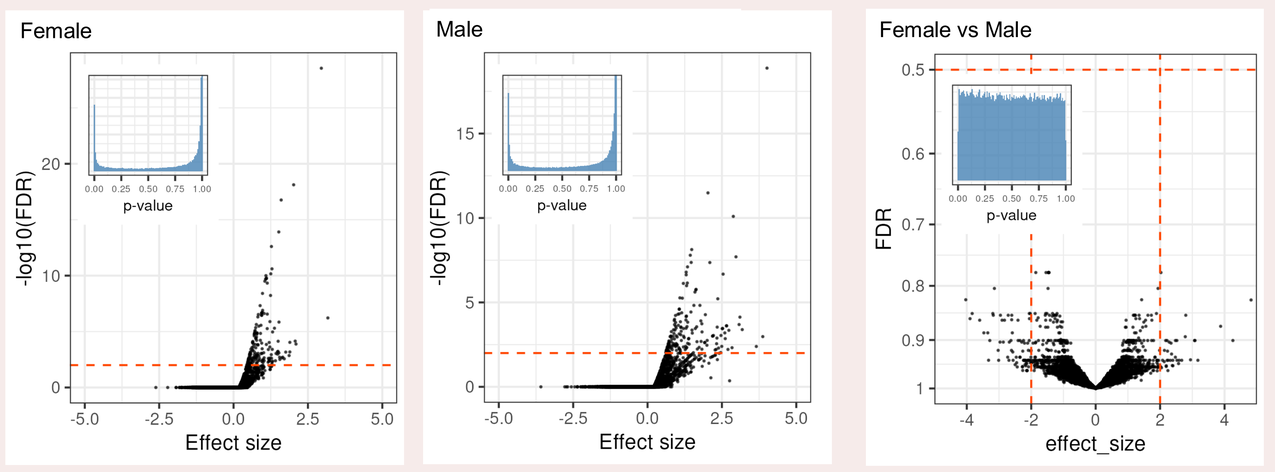
Output
edge- and gene-level differential wiring:
interpretable summaries across genes and MSigDB[3] gene sets (PID collection)

*__sample__: data of a single donor / patient / tissue sample in one cell type
**__edge__: gene – gene connection

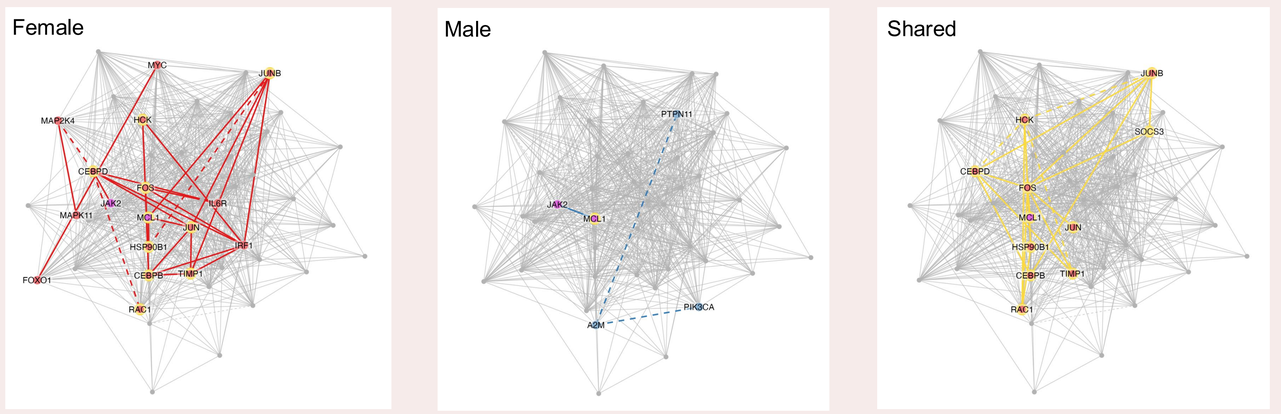
4 Results

One-group tests
$H_0: \beta \leq \text{min\_effect\_size}$

Two-group test
$H_0: \beta_1 = \beta_2$

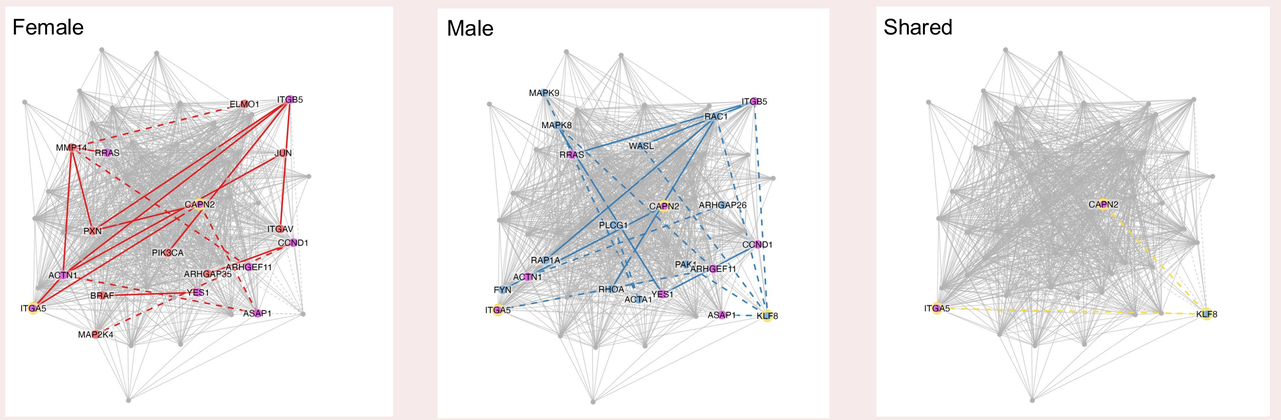


PID_IL6_7_PATHWAY — Example of a pathway with conserved core: large fraction of shared edges (right panel)



Shared network points to coordinated early inflammatory transcription centered on AP-1 factors (FOS/JUN/JUNB), with female-specific edges connecting into this module, particularly through IRF1.

PID_FAK_PATHWAY — Example of a pathway with dominating group-specific wiring of edges



Differential wiring suggests altered coordination of adhesion, cytoskeletal, and migratory machinery: females show hubs around ACTN1 / MMP14 and integrins, while males show actin-signaling connectivity via KLF8 / RAC1 / PAK1 / WASL.

Edges
- Female
- Male
- Shared
- Female diff.
- Male diff.
- STRING-db
- Non-STRING-db

Genes — node colors determined by edge connections
- Female
- Male
- F & M
- Shared (S)
- Female & S
- Male & S
- F & M & S
- STRING-db

Reading example: ITGA5 is purple, as it is connected Female- and Male-specific edges, and has yellow rim, as it also has a shared edge, RAC1 is blue due to exclusively Male (blue) edges.

5 References

1. Langfelder & Horvath, *BMC Bioinformatics* **2008**, 9:559
2. Ritchie et al., *Nucleic Acids Research* **2015**, 43:e47
3. Liberzon et al., *Bioinformatics* **2011**, 27:1739–1740.
4. Yin et al., *Science* **2026**, 391:eadt3130