Presentation 7956

Immune profiling in Sjögren's Disease: Insights from Single-Cell RNA sequencing of PBMCs

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Introduction

Sjögren's Syndrome (SS) is a chronic autoimmune disease characterized by the immune infiltration of the salivary and lacrimal glands, leading to hallmark symptoms such as dry mouth and eyes. While immune cell dysregulation is central to SS pathogenesis, further research is needed to comprehensively characterize these changes at the cellular and molecular levels.

Objective

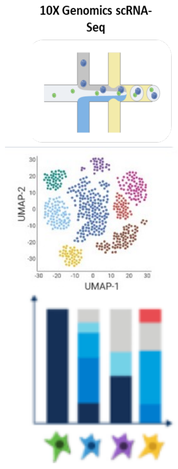
To perform a comprehensive single-cell RNA sequencing (scRNA-seq) analysis of peripheral blood mononuclear cells (PBMCs) from SS patients and healthy controls, to investigate disease-associated changes in immune cell composition and gene expression.

Methods

PBMCs sc-RNA-seq 3´ 10X Genomics protocol

1. SS* data generation*:

SSAD cohort
- 32 primary SS and
- 10 matched healthy (H)
(198,078 cells).



2. Downstream analysis :

- Mapping reads to reference genome.
- Quantification of gene expression.
- Reference-based cell annotation (automatic and manual).

-Statistical analyses: Cell composition, Gene expression.

Results

Multivariate statistical comparisons of cell type abundance (Fig. 1.A) of SS vs H samples revealed significant differences: **Increase in SS** of **CD8+ T_{EM}**, **CD4+ TM.Th2** and **proliferative TNK** cells. **Decrease** of **naïve CD8+ T, naïve CD4+ T, MAIT, pDC, DC3, B intermediate** cells (Fig. 1.B). Compartment-specific (TNK, myeloid, B separately) Covarying Neighborhood Analyses $(CNA)_1$ detected a subpopulation within the TNKs of **CD8+ T_{EM}** cells overrepresented in SS (Fig. 1.C).

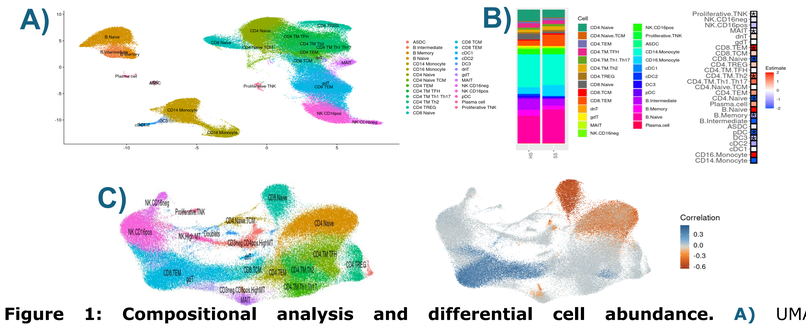


Figure 1: Compositional analysis and differential cell abundance. A) UMAP representation of the transcriptome of 198,602 PBMCs. **B)** Graph depicting comparison of relative cell abundances (left) and results from the statistical comparisons of SS vs H abundances. Positive estimates (coefficients obtained from the analysis) represent higher abundance in SS compared to H . **C)** CNA analyses in the TNKs detected a subpopulation within the TNKs of CD8+ T_{EM} cells overrepresented in SS (significant positive correlation, in blue). No significant results were obtained in myeloid or B compartments.

The subpopulation of CD8+ T_{EM} cells enriched in SS has DE genes linked to SS $risk_2$ (Fig. 3.A). We found a significant enrichment of DE genes in the SS-linked gene set (Fig. 3.B).

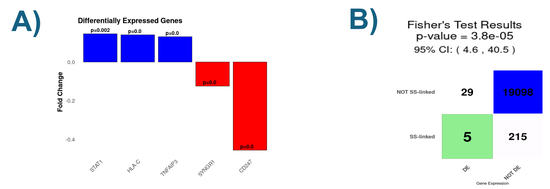


Figure 3. Genes linked to SS risk differentially expressed in the subpopulation of CD8+ T_{EM} cells enriched in SS. A) Average log Fold-Change values and P-values obtained from Differential Gene Expression Analyses with MAST. Positive log Fold-Change values are obtained if the gene is overexpressed in this cell population. **B)** The amount of these genes is significantly enriched, according to a Fisher Exact Test.

SS drives pervasive changes in the gene expression profile of PBMCs. Differential gene expression analyses revealed that Monocytes and NK CD16+ cells express the highest number of differentially expressed (DE) genes compared to controls. Also, CD14+ Monocytes and cDC2 display the highest overactivation signal (Fig. 2.A). We found a general immune activation in SS across all major PBMC compartments. For instance, our results show upregulation of pathways related to interferon I and II (Fig. 2.B)

Figure 2. SS-driven differentially expressed genes and pathways. A) Ratio of over-expressed vs under-expressed genes (SS vs H) in PBMCs. Circle size represents number of DE genes and color represents ratio of down-regulated vs up-regulated genes (lower values when more up- vs down-regulated genes). **B) Gene Ontology (GO) enrichment analyses** of pathways related to interferon I and II in the PMBCs. Larger circle sizes represent more significant P-values.

Conclusions

- This study provides the largest scRNA-seq analysis of PBMCs in SS to date. We have generated a comprehensive atlas mapping **~200,000 single cells from SS PBMCs** to assess SS transcriptomic signatures compared to healthy donors.
- We have identified **Monocytes** as the circulating cells with the largest fraction of dysregulated genes, supporting their key role in SS.
- We have detected a subpopulation of **CD8+ T_{EM}** cells overrepresented in SS, characterized by an enrichment in genes associated to SS risk.

References:

1- Reshef, Y. *et al*. (2022). Covarying neighborhood analysis identifies cell populations associated with phenotypes of interest from single-cell transcriptomics. *Nature Biotechnology*, 40(3): 355-363.

2- Khatri, B. *et al.* (2022). Genome-wide association study identifies Sjogren's risk loci with functional implications in immune and glandular cells. *Nature Communications*, 13(1): 4287.

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SSAD Síndrome de Sjögren

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