Elucidating the Roles of Subtypes of B cells and Fibroblasts in the Immune Response of Rheumatoid Arthritis Joint Synovial Tissue

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Abstract- **Rheumatoid Arthritis (RA) is an autoimmune disorder characterized by inflammation, while Osteoarthritis (OA) is developed through wear and tear on the body. B cells and Fibroblasts are activated by inflammation, however, the roles of subtypes of these cells in the progression of RA are unclear. Through single cell RNA sequencing on a dataset with RA and OA, the B4 subtype of B cells and the F2 subtype of fibroblasts were identified. The Immunoglobulin Heavy Constant Gamma 3 (IGHG3) gene, which codes for an immunoglobulin protein, was upregulated, suggesting its potential as a marker of RA. Through gene set enrichment analysis, upregulated pathways of the subtypes were identified. Immunoglobulin related pathways were upregulated in the F2 subtype suggesting that F2 cells are a crucial part of the immune response. Interferon related pathways were enriched in B4 cells suggesting that B4 cells regulate immunoglobulin class switching to Immunoglobulin G (IgG) molecules. The functions of the F2 and B4 subtypes are unique to RA and provide new information that is necessary to further elucidate the roles of B cells and Fibroblasts in RA.**

I. Introduction

Osteoarthritis (OA) and Rheumatoid Arthritis (RA) are two forms of arthritis. While OA occurs due to wear and tear on joints, RA is a chronic autoimmune disease characterized by inflammation and swelling of the synovial membrane in joints with eventual tissue and bone damage. Early detection and treatment are crucial for positive prognosis. [1]

Autoantibodies are produced by the body during RA against the body's antigens, which results in an aggressive immune response that destroys the cells, tissue, and eventually bones of the body. The inflammatory response is composed of immune cells, such as B cells and T cells, as well as fibroblasts and monocytes. [2] Currently researchers are focusing on key cells, such as B cells, T cells, Fibroblasts, and Monocytes that are involved in RA pathogenesis as potential targets. However, there are many subtypes for each of these cells, whose role in RA are unknown. The subtypes of B cells and fibroblasts were investigated using single cell RNA sequencing and gene set enrichment analysis to understand the dysfunctional immune response and the mechanisms that can be targeted to develop more efficient treatments and markers for RA. [1],[2]

II. Methods

Analysis of single cell RNA-sequencing was performed on a dataset of Rheumatoid Arthritis and Osteoarthritis, which included aligned and mapped data from the joint synovial tissue samples of n=30 human patients (20 RA and 10 OA). Seurat, dplry, ggplot2, and cowplot packages were downloaded into Rstudio to analyze and visualize the data. The data underwent quality control, reduction of dimensions, and then visualization of clusters with a Uniform Manifold Approximation and Projection (UMAP) graph. [3] The data was divided into four Seurat objects based on cell type: B cell, T cell, Fibroblasts, and Monocytes. The same pathway of analysis was used to identify clusters that represented the subpopulations of each cell type with a UMAP visualization. Each cluster was named to represent the cell type they were related to: B cell subtypes were referred to as B0-B5; Fibroblast subtypes were labeled F0-F5. Volcano plots and dot plots of the Seurat objects were also created in RStudio to identify differentially expressed genes in each group.

Gene Set Enrichment Analysis (GSEA) was utilized to define enriched pathways [3]. The first comparison was between RA and OA. The next two comparisons only included B cells and Fibroblasts, respectively, and was a comparison between RA and OA for each cell type. The last comparison was between a subpopulation and the rest of the subpopulations for the B4 subtype and the F2 subtype respectively. The c5 gene set database from the Molecular Signatures Database was used as the reference database for each GSEA. The GSEA software calculated statistical significance for each analysis and that data was used to determine which pathways were significantly unregulated. Enrichment analysis was considered statistically significant when the nominal p-value cutoff < 0.05 [3]. Enriched pathways from each of the GSEA results were combined into a bar graph with the normalized enriched scores (NES) to visualize the results.

III. Results and Discussion

Fibroblasts

The UMAP image shows the clusters of the subpopulations of fibroblasts split by condition (Fig 1A). F2 cells were only present in RA patients and made up 12.39% of the fibroblast population (Fig 1B). GSEA results indicated that immunoglobulin related pathways were enriched only in the F2 subtype (Fig 1C). F2 cells express IGHG1 and IGHG3 genes (Fig 1D).
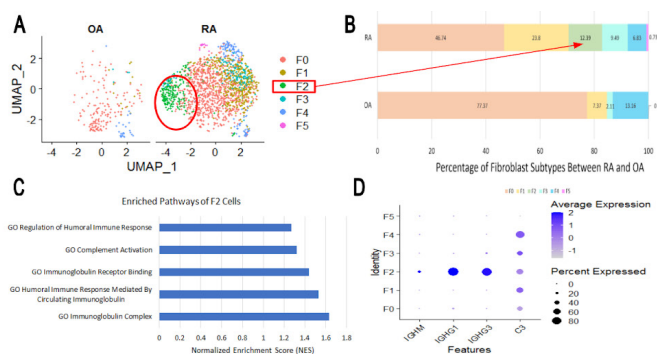

Figure 1: Differences of Fibroblast subtypes between OA and RA. **A.** The UMAP Visualization of the Fibroblast subtypes. **B.** The percentage of each subtype between conditions shows that F2 cells are unique to RA. **C.** Enriched Pathways in F2 Fibroblasts when compared to the rest of the fibroblast subtypes. **D.** Dotplot of the four core genes in Fibroblasts related to the GO Humoral Immune Response Pathway from C. (Figures by author)

The enriched complement activation pathway indicates that F2 fibroblasts directly promote inflammation through the creation of immunoglobulin immune complexes with fibroblasts (Fig 1C). F2 fibroblasts may be antigen presenting cells due to the gene expression levels of IGHG1 and IGHG3 (Fig 1D). [4]

B cells

The UMAP image shows the clusters of the subpopulations of B cells split by condition (Fig 2A). The bar graph shows that B4 cells were only present in the RA condition and made up 5.65% of the B cell population (Fig 2B). GSEA results indicate that interferon related pathways are enriched in B4 cells compared to the other B cell subpopulations (Fig 2C). B2 and B3 cells express IGHG2, IGHG3, and IGHG4 genes (Fig 2D).
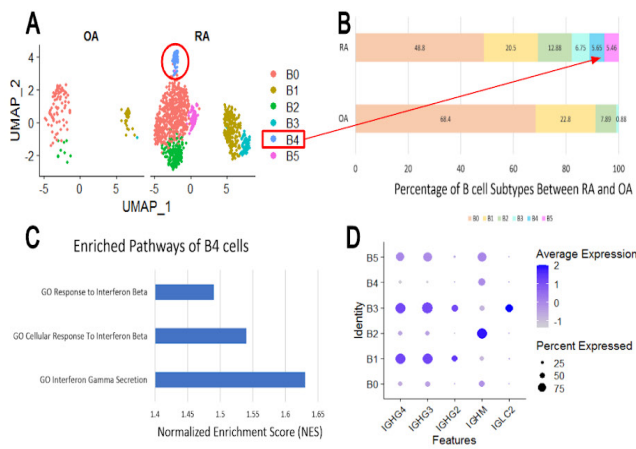

Figure 2: Differences of B cell subtypes between OA and RA. **A.** The UMAP Visualization of the B cell subtypes. **B**. The percentages of each subtype between conditions **C.** Enriched Pathways of B4 cells when compared to the rest of the B cell subtypes. **D.** Dot plot of the five core genes related to the Go Immunoglobulin Complex pathway. (Figures by author)

Interferon gamma secretion pathway was enriched in B4 cells. B cell subpopulations can produce IFN-γ in immune responses, such as the immune response in RA [5]. (Fig 2C) Interferon gamma (IFN-γ) has the ability to promote immunoglobulin class switching in B cells; IFN-y promotes IgG class switching to a more pathogenic subclass, IgG3, in RA [5]. This suggests that B4 cells are indicators of disease severity of RA and are essential to immunoglobulin class switching. Additionally, two pathways were enriched in B4 cells that indicated a role in responding to interferon beta. (Fig. 2C) Previous research has suggested that interferon betas have an anti-inflammatory role in the pathogenesis of RA [6].

IV. CONCLUSION

Immunoglobulin upregulated pathways and the presence of IGHG3 in both B cells and Fibroblasts indicates that the IGHG3 gene may be a potential biomarker for RA [7]. The F2 subtype provides evidence that there is a strong link between the immune response and fibroblasts through immunoglobulins [5]. The B4 subtype showed evidence of an important role in immunoglobulin class switching to igG and

specific IgG1-4 molecules. This function has a direct link to disease severity as class switching of Igs indicates disease progression [1]. This relationship between B subtypes and immunoglobulins may uncover the possibility of dependent interactions between the functions of fibroblasts and B cells, and may lead to the development of a new treatment that can target these pathways of disease progression.

VI. REFERENCES

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