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Transitional B cells in PBMCs and its pathogenesis in SLE using sc-RNA sequencing data

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Abstract. Systemic lupus erythematosus (SLE) is a autoimmune disorders with multisystems and organs such as kidney, lung and heart et. al. The abnormal functions of B cells induced the pathogenesis and pathological progression of this disease. The therapeutic strategies targeting B cells have been used clinically to treat SLE, achieved encouraging result. However, some patients have side effects such as infection caused by excessive humoral immune suppression. Sc-RNA sequencing technology can sequence the overall RNA in the level of single cell, aiming to classify cells, thus achieving to target B cell subgroups precisely. This article utilized transcriptome sequencing data of single cell from PBMCs in the GEO database, and subsequently analysed for dimensionality reduction clustering and data screening using R packages. Ultimately, the author discovered highly variable genes and related pathways of SLE samples' transitional B cells, providing possible directions to explore SLE related treatment candidates in future.

Keywords: systemic lupus erythematosus (SLE), sc-RNA seq, transitional B cells.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorders with multisystems and organs such as kidney, lung and heart et. al. [1]. The abnormal functions of B cells induced the pathogenesis and pathological progression of this disease. The therapeutic strategies targeting B cells have been used clinically to treat SLE, achieved encouraging results including decreasing the level of auto-antibodies and reducing infiltration of inflammation. For example, the monoclonal antibody of rituximab binds to antigen CD20 which is specific in the B-cell, making an effects of depleting B cells from the peripheral blood and lymphoid tissues. However, some patients have side effects such as infection caused by excessive humoral immune suppression, suggesting that targeting pathological B cell subsets possibly resolve the associated problems, such as targeting autoreactive B cells.

Sc-RNA sequencing technology can sequence the overall RNA in the level of single cell, aiming to classify cells, and discover the processes of cell evolution and new subgroups with unknown function, thus achieving to target B cell subgroups precisely. Previous report showed that designed CARs targeting autoreactive B cells, which have displayed optimistic effects in the treatment of SLE, but is still in the preclinical research stage in this field [2]. The pathological autoreactive B cells in PBMCs of SLE patients derived from the transitional B cell subpopulations during the developmental stage of bone marrow B cells [3]. However, there are still many unclear mechanisms regarding the characteristics of this group of cells and how they evolve into pathological autoreactive B cells involved in the occurrence

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and progression of SLE. Therefore, it is necessary to have a deeper understanding of the characteristics of transitional B cells and their roles in SLE. This article utilized transcriptome sequencing data of single cell from peripheral B cell cells in the GEO database, and subsequently analysed for dimensionality reduction clustering and data screening using R packages. Ultimately, the author discovered highly variable genes and related pathways in transitional B cells in SLE samples, providing possible directions to explore SLE related treatment candidates in future.

2. Methods

2.1. Data source

Two groups of single-cell sequencing data of SLE patients' peripheral blood B cells and normal controls, GSE242615 (SLE patients, n=33695) and GSM6619417_C001 (control group, n=36602), were downloaded respectively from the GEO database. The peripheral B cells preparation method from SLE patients was as follows: FACS-selected $CD19^+$ B cells from PBMC of patients with newly diagnosed SLE were re-suspended in PBS and the survival rate of isolated cells was >90%. Then, to prepare a library, a 10x Genomics Chromium single-cell 5 'v2 chip was used for loading a single-cell suspension. The quality of the library was examined by Agilent 2100 bioanalyzer and Qubit fluorescence quantification. The samples were sequenced on the platform of Illumina NovaSeq 6000 and each cell have an over 50,000 sequencing depth reads.

Healthy control peripheral B cells were obtained in the following way: firstly, Ficol-Hypaque density gradient centrifugation was used to isolate the peripheral blood samples. Subsequently, using B cell isolation Kit II, through negative selection, B cells were separated from PBMC, in which non-B cells were labeled with biotinylated antibodies, and then magnetically labeled with anti-biotin MACS microbeads which were beneficial for negative sorting using LD column and magnetic bead separator.

Both the sequencing platform of these two groups were GPL24676Illumina NovaSeq 6000. The same sequencing platform reduced noise differences and control variables.

2.2. Statistical software

The quality control method was used in the base package to screen two sets of peripheral B cell data and select higher quality cells with deleting an appropriate number of RNA features (200 to 2500) and a low proportion of mitochondria (less than 5%). Then the RunPCA function in the Seurat package was used for principal component analysis and the DimHeatmap function was used to find the characteristic genes of B cells. Next, three functions, FindNeighbors, FindClusters and RunUMAP from the Seurat package, were used to cluster SLE and healthy peripheral B cell data in dimension reduction. The parameter resolution was 0.5 and 0.3 respectively, and 7 different types of B cells were generated involving transitional B cells, naive B cells, activated naive B cells, age-associated B cells (ABCs), memory B cells, plasma blast cells, and plasma cells. The ggplot2 package was used to create the umap figure. Finally, clusterProfiler and org.Hs.eg.db, these two packages were used to convert the hypervariable genes in B cells from symbol ID to ENTREZ ID which was prepared for GO and KEGG enrichment analysis.

3. Results

3.1. Screening B cells in PBMCs

First of all, to verify that the selected data is B cells, quality control of the data, searching for highly expression genes, scaling the data, and PCA processing were performed to find the genes with the highest expression. The data showed that BCL7A, BACH2, FCER2, VPREB3, CD23 and other genes specifically or mainly expressed in B cells. The appearance of them in hyperactivation demonstrated that the data study was indeed B cells with a heatmap shown in Figure 1.

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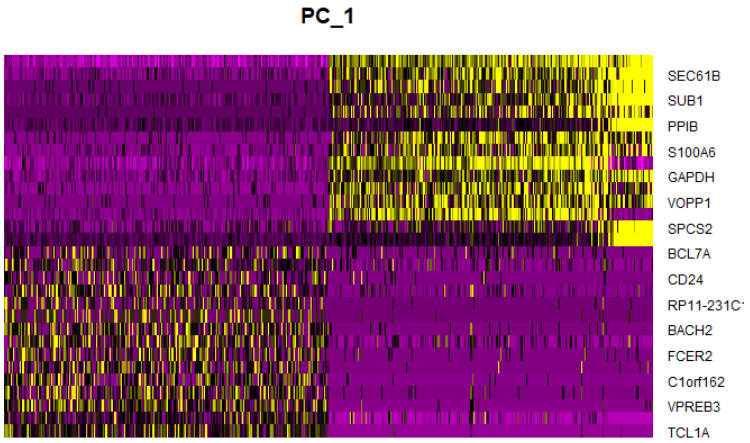

Figure 1. Heat map of marker genes in B cells.

3.2. Changes of B-cells in SLE patients compared with control group

After filtering peripheral blood B cell data, they were subjected to dimensional-reduction clustering. Seven clusters were obtained through umap dimensional-reduction algorithm. These cluster names were also added to the umap map which helped making comparison. The number of B cells group expression changed between the SLE patients and healthy group by comparing the two umaps. Among seven clusters, transitional B cells are the cluster of interest in this paper, as shown in Figure 2-3.

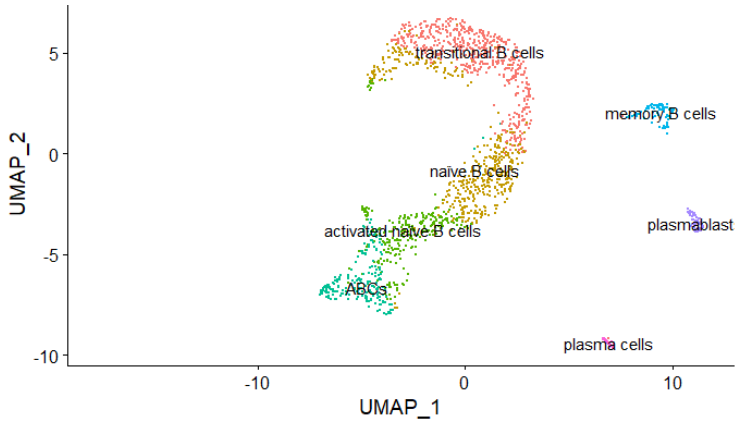

Figure 2. Dimensionality reduction cluster of B cells in SLE patients.

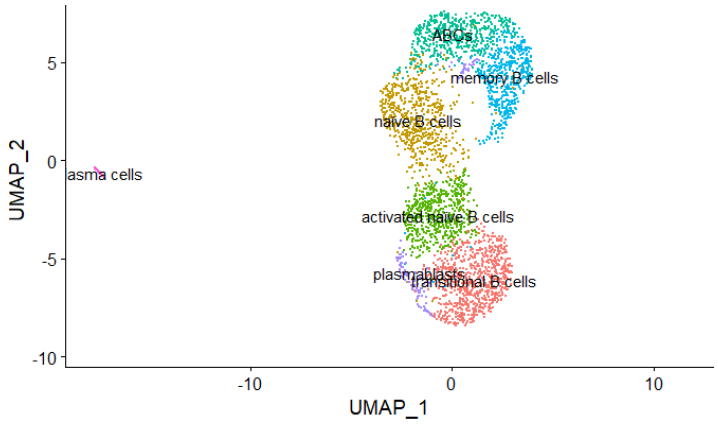

Figure 3. Dimensionality reduction cluster of B cells in the healthy control group.

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Further analysis of SLE patients and the healthy dataset showed significant proportion of different cell clusters changes between SLE patients and healthy subjects. The radio of transitional B cells increased from 20.99% to 30.43%, and plamablasts from 5.99% to 34.47% in SLE patients (Figure 4). These changes demonstrate significant differences at the cell subset level between SLE patients and healthy people.

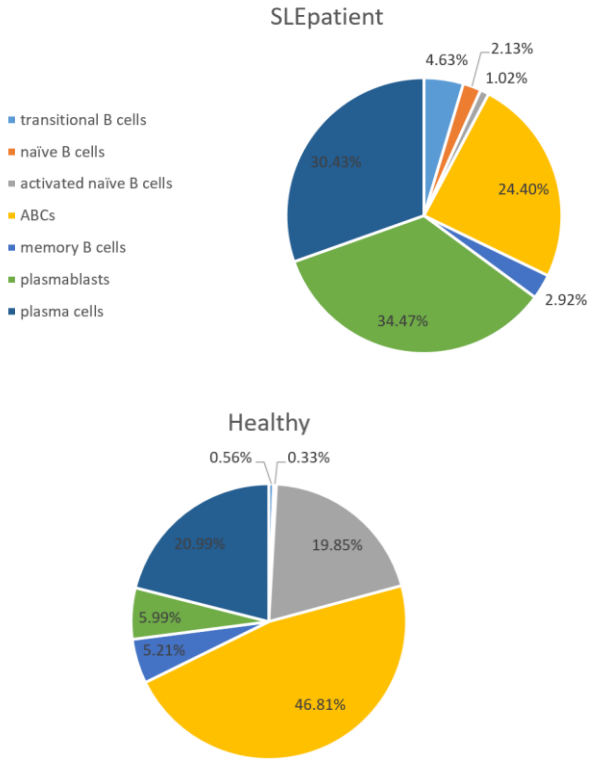

Figure 4. Pie chart of B cell proportion in SLE and healthy control.

3.3. DEGs in transitional B cells

All transitional B cell genes were first screened from all B cells, and then through differential gene analysis method, the hyperactivated top 10 genes in transitional B cells were filtered. Based on the expression of the top 10 genes in different clusters, heat maps were made, as shown in Figure 5.

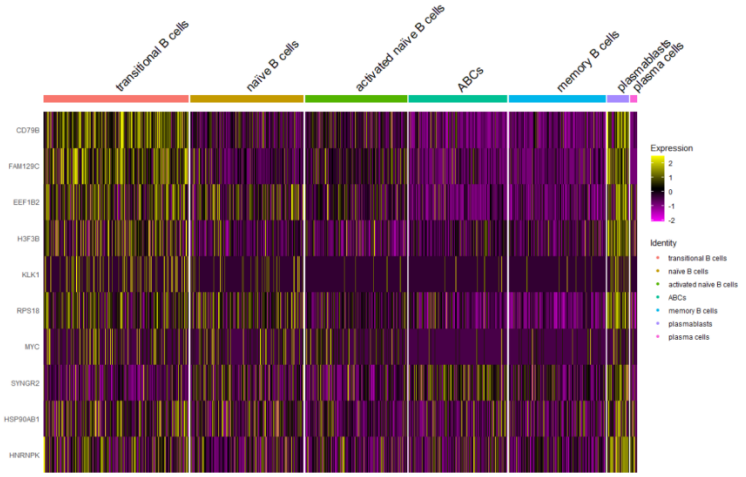

Figure 5. Heat map of top 10 genes expression in different cluster groups.

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3.4. Enrichment analysis of GO and KEGG pathway

Finally, all the highly variable genes in SLE patients' transitional B cells, a total of 76, were filtered for enrichment in the GO and KEGG pathway. Both the two enrichment methods' result showed two most highly expressed pathways, such as pathways of Ribosome and Coronavirus disease of COVID-19 (Figure 6-7).

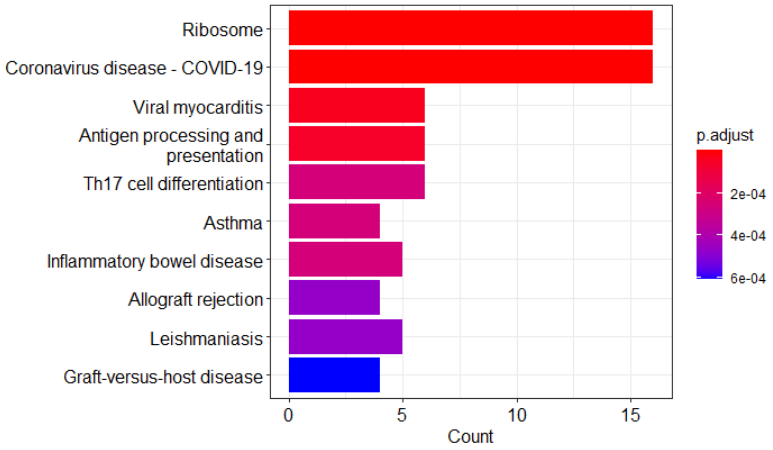

Figure 6. GO pathway enrichment analysis in high variable transitional B cells of SLE patients.

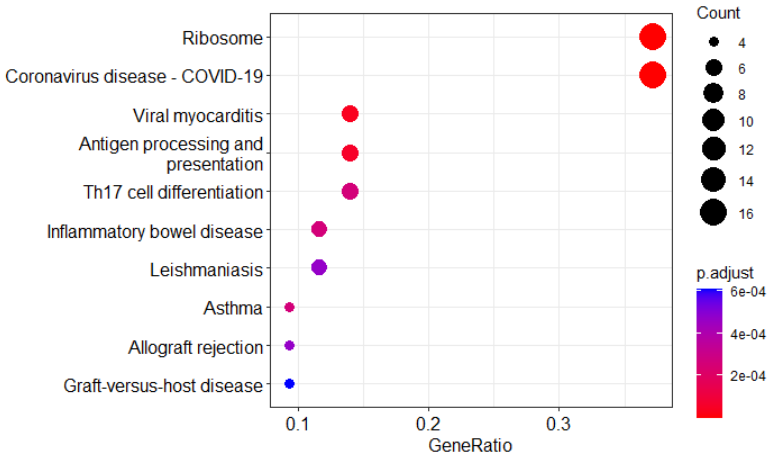

Figure 7. Dot plot of KEGG enrichment analysis of high variable transitional B cells of SLE patients.

4. Discussion

By comparing the umap data of SLE and healthy people, it can be seen that a large number of changes have taken place in different categories of peripheral B cells. The proportion of each cell subpopulation has changed significantly. In these seven peripheral B cells' clusters, transitional B cells changed from 20.99% to 30.43%, plamablasts from 5.99% to 34.47%. Previous study showed that obvious abnormality in the ratio of lymphocytes' subgroups in SLE patients in comparison to RA patients or healthy controls (both $P < 0.01$), consistent with this study [4].

According to the changes of B cell subsets' proportions in SLE patients and healthy people in umap data, transitional B cells increased their proportion, which is consistent with previous studies. Data from Dieudonné's group showed that the upregulation of frequencies in transitional B cells, as the main pathogenic B cells derived from immature B cells in bone marrow, were positively correlated with disease severity. In addition, this groups discovered that the expression of CD19 was downregulated and TLR9 signal pathway was defected in SLE transitional B cells [5]. However, it remains uncertain what

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pathway SLE is involved in affecting transitional B cells, causing their defects, eventually exacerbate the development of SLE.

As the most immature B cells, transitional B cells need to express a large number of genes to realize the function of mature B cells or become autoimmune associated B cells. The upregulation proportion of transitional B cells in the subpopulation of SLE patients suggested its pathological functions with activation of Ribosom-related pathways in this study.

As for the second COVID-19 pathway, its mechanisms are still being explored. Some individuals suffered COVID-19 were then more prone to SLE and varicella-like rash [6]. This possible explanation is the virus infection activates the autoimmune response of susceptible individuals, causing them to develop autoimmune diseases, such as SLE. Acute COVID-19 infection have a relationship with increased peripheral B cells and activation of the antiviral immune response. A large number of proinflammatory cytokines are produced, which makes COVID-19 likely to induce SLE because an uncontrolled proinflammatory cytokine. In addition, the chronic autoimmune disease of SLE patients were more susceptible and vulnerable to virus infection such as COVID-19 due to immunosuppression since they treated with immune-suppressants [7]. Due to the similar immune characteristics between SLE and this virus infection, such as the higher immune response, inflationary reactions and cytokines storm, providing similar candidates in understanding the pathogenesis and treatment targets for both diseases [8]. However, the similar genes and mechanisms between SLE and COVID-19 require further analysis.

5. Conclusion

In conclusion, the author screened transitional B cells which increased 10% in SLE patients compared to control, suggesting this subgroup possible involved in the occurrence and development of SLE. Therefore, the changed genes and their related pathways was analyzed to understand the pathogenesis in SLE using sc-RNA sequencing data.

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