Immune Infiltration Characteristics of Related Receptor Signaling Pathways in Primary Sjögren's Syndrome

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Research

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Abstract

Background: Primary Sjögren's Syndrome (pSS) is a chronic systemic autoimmune disease characterized by a broad spectrum of clinical features. It is considered to be associated with immune cells and genetic. Since the pathogenesis of pSS has not been studied thoroughly enough, it is significant to explore the relevant mechanisms using bioinformatics methods.

Methods: We downloaded the GSE84844, GSE66795 and GSE51092 datasets from the GEO database, and then conducted a comprehensive bioinformatics analysis including differentially expressed genes (DEGs), functional enrichment pathways and immune infiltration characteristics.

Results: DEGs analysis identified a total of 89 up-regulated genes and 11 down-regulated genes in the dataset. These DEGs were enriched in NOD-like and RIG-I-like receptor signaling pathway, which were significantly associated with the expression of immune cells such as neutrophils and activated dendritic cells, respectively.

Conclusion: The NOD-like and RIG-I-like receptor signaling pathway and the pathogenesis of pSS may be closely associated. Neutrophils and dendritic cells also play an important role in pSS, and the expression of these two kinds of cells is closely associated with the signaling pathways of NOD-like and RIG-I-like receptors.

Introduction

Primary Sjögren's Syndrome (pSS) is a chronic systemic autoimmune disease characterized by a broad spectrum of clinical features(1–3). This disease can result in dryness of major mucosal surfaces, such as mouth, eyes, nose and throat(4). pSS primarily affects women in middle age, in a 9:1 ratio of women to men and an estimated prevalence of 0.3-3 per 1000 in the general population(1, 5). There is a 6.5-fold increased risk of non-Hodgkin's lymphoma in patients with pSS(6, 7). In addition, unlike secondary Sjogren's syndrome (SS), pSS can occur alone. The development of secondary SS is associated with autoimmune diseases, for instance, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (8).

Since it is a complex and multifactorial disease, a range of environmental and genetic factors are involved in the initiation and procession of pSS. As an autoimmune disease, related studies point out that the typical histological features of pSS are the infiltration of immune cells in the lacrimal and salivary glands, such as T cells, B cells, macrophage and dendritic cells (DCs)(9). Some studies in patients with pSS suggest that there may be various disruptions in the differentiation and maturation of B cells, such perturbations including altered transport of B cells between the peripheral circulation and inflammatory glands, impaired differentiation of B cells and more production of self-activating plasma B cells in circulation. The overactivity and dysregulation of B cells seems to be a signature of this disease(10–13). Distinct CD4+ T cell subsets may also be associated with autoimmune activity in pSS. This natural

balance between different CD4+ T cell subsets, namely Th1, Th2, Th17, follicular helper T cells (Tfh) and regulatory T cells (Tregs), is disrupted in pSS patients(14, 15).

However, because of the overly complex clinical phenotype and pathogenesis of pSS, its specific etiology currently needs to be explored in greater depth. Therefore, it is necessary to identify disease-associated biomarkers to understand the complicated progression of pSS. With the advancement of next-generation sequencing technology and the improvement of biological databases, it is significant to explore the relevant mechanisms using bioinformatics methods. We merged the gene expression omnibus (GEO) datasets from 3 pSS studies, then performed specific bioinformatics analysis, including differentially expressed genes, functional enrichment pathways, immune infiltration characteristics.

2 Materials And Methods

2.1 GEO datasets

The microarray datasets for this study on pSS were obtained from GEO (https://www.ncbi.nlm.nih.gov/geo). GEO serves as an international public repository for the research community to share data in multiple formats, including primarily microarrays and other forms of high-throughput functional genomics data. The following keywords were used to locate the GEO dataset consistent with the purpose of the study: 'primary Sjögren's Syndrome' MeSH Terms AND 'Expression profiling by array' DataSet Type AND 'Homo sapiens' Organism. Referring to the mentioned conditions, we finally screened three pSS datasets including GSE84844, GSE66795 and GSE51092, and table 1 shows the basic information of the datasets.

Table 1

Basic information about datasets

Dataset	Platform	Sample	pSS cases	Health control	Gene type
GSE84844	GPL570	peripheral blood	30	30	RNA
GSE66795	GPL10558	peripheral blood	131	29	RNA
GSE51092	GPL6884	peripheral blood	190	32	RNA

2.2 Dataset consolidation and de-batching effects

We used the annotation file to annotate each of the three downloaded gene matrix files, and corresponded the probe IDs in the matrices to the gene symbols to finally obtain the three gene expression matrices. These expression matrix datasets were then merged into one. The R package "sva" installed from Bioconductor (https://bioconductor.org/) was used to remove the heterogeneity caused by different experimental conditions, environments, personnel, and other factors.

2.3 Data preprocessing and differentially expressed genes (DEGs) analysis

The R package “limma” was applied to screen DEGs in cases and healthy controls, and P values were modified according to the Benjamini and Hochberg’s method. We set logFC greater than 0.5 while adjusted P value < 0.05 between the two groups was regarded as significantly different. The volcano maps and heat maps were created using Sangerbox Tools (http://www. sangerbox. com/tool).

2.4 Gene ontology (GO) and pathway functional enrichment analysis

After identifying the up- and down-regulated genes in the pSS, the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional classification tool was applied to define biological functions for these genes, including the cellular components (CC), biological process (BP), molecular function (MF). Meanwhile, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was also done using this tool. The thresholds for GO analysis were determined as enrichment score > 1.0 and P < 0.05. The threshold for KEGG pathway analysis was P < 0.05.

2.5 Protein-Protein interaction networks (PPI) and Hub genes

STRING (https://www. string-db. org) is an online data analysis tool for building PPI networks of cases and healthy controls. Cytoscape is a publicly available software platform for visualizing complicated networks and combining these networks with any kind of data. The PPI network was plotted using Cytoscape3.8.2. Hub genes are key genes in protein functional network. They were screened and mapped using the Degree method with CytoHubba package of Cytoscape.

2.6 Gene set enrichment analysis

We used R to implement the Gene set variation analysis (GSVA) process. GSVA is a GSE method for estimating changes in pathway activity in a sample group. R was employed to analyze KEGG-enriched pathways with cut off values set to logFC > 0.15 and adjusted P value < 0.05. In addition, Gene set enrichment analysis (GSEA) was used to explore the immune-related signaling pathways enriched in pSS and simultaneously visualize the GSEA results. The process was done using the GSEA 4.1.0.

2.7 Composition analysis of immune cells

Cibersort is an algorithm that analyzes the type and distribution of various immune cells in a sample by calculating the differential expression of marker genes in different immune cells of the RNA transcript(16). We used this algorithm to assess the expression levels of 22 kinds of immune cells in peripheral blood samples from pSS patients and controls. The process achieved using R package “Cibersort”. Visualization of Cibersort results was also done through a series of R packages, including “MatrixStats”, “PheatMap”, “RcolorBrewer”, “TidyVerse”, “Cowplot”, “GGPUBR”, “Bslib” and “GGthemes”.

2.8 Correlation analysis of immune cells and pathways

To explore the correlation between pathways and immune cells pathways, we used the method of Spearman correlation analysis, and the threshold was set at $P < 0.05$. Graphs were constructed with Graphpad 8.0.

3 Results

3. 1 Data integration and removal of batch effects

It is easily seen from Figure 1A that after normalizing the data from different sources, the overall expression distribution of the samples is homogeneous without the batch effect. Meanwhile, we identified 10367 genes in the merged dataset (Figure 1B).

3. 2 Differentially expressed genes in pSS

The dataset included 351 pSS patients and 91 healthy controls. Analysis of variance revealed 100 differential genes in the merged dataset, including 89 up-regulated genes and 11 down-regulated genes. The results of DEGs are presented in the form of heat maps and volcano maps (Figure 2).

3. 3 Functional annotation and pathway enrichment analysis

The results of GO analysis by DAVID showed that DEGs in pSS were mainly variations in MF, including double-stranded RNA binding and single-stranded RNA binding, etc. (Figure 3A). BP variations were enriched considerably in response to virus (Figure 3B). KEGG pathway analysis showed that the main enrichment pathways for DEGs in the immune system were the NOD-like and RIG-I-like receptor signaling pathway (Figure 3C,3D).

3. 4 PPI network analysis and hub gene selected

After eliminating isolated genes, the PPI network was generated with Cytoscape (Figure 4A). Further analysis of the PPI network by Cytohubba identified a total of 10 hub genes, which are at the center of the graph (Figure 4B).

3. 5 Gene set enrichment analysis in pSS

Different from GO and KEGG analysis, GSEA is a method for analyzing genes in comparison to a predefined set of genes from the perspective of their enrichment without the need to determine a threshold. Gene expression profile data are analyzed to understand their expression status in a specific set of functional genes and whether there is some statistical significance in this expression status. Therefore, we used GSEA to explore biological variations between pSS and normal individuals. The same as KEGG results, GSEA also showed that both NOD-like and RIG-I-like receptor signaling pathway are abnormally regulated in the immune system of pSS (Figure 5).

3. 6 Immune infiltration analysis

According to the results of Cibersort, a variety of immune cells are differentially expressed among the 22 kinds of immune cells between the cases and controls (Figure 6). Immune cells with significantly different expression in the two groups include resting NK cells, activated dendritic cells and neutrophils, in addition to some B cell subsets and T cell subsets.

3.7 Correlation between signaling pathways with immune cells

In order to further explore the association between the pathways and immune cells, we applied the GSVA enrichment score of the signaling pathway and immune cell infiltration score for spearman correlation analysis. The results showed that NOD-like receptor signaling pathway was correlated significantly with 9 kinds of immune cells and RIG-I-like receptor signaling pathway was correlated with 6 types immune cells (Figure 7). In addition, there are 4 significantly related immune cells are common to both pathways, namely neutrophils, activated DCs, resting NK cells, and memory B cells (Table 2). The scatter plots indicate that activated DCs and neutrophils are closely associated with both two immune signaling pathways (Figure 8).

Table 2

Correlation between pathways and immune cells

Cells	Pathways			
	NOD-like		RIG-I-like	
	R	P value	R	P value
Memory B cells	-0.0978	0.0399	-0.1369	0.0039
Resting NK cells	-0.1143	0.0162	-0.1428	0.0026
Activated DCs	0.3230	<0.0001	0.4022	<0.0001
Neutrophils	0.4300	<0.0001	0.4562	<0.0001

* R, Spearman correlation coefficient value.

4 Discussion

In order to expand the sample size, we merged the three datasets and finally obtained the gene expression data of 351 cases and 91 healthy controls, which provided a reliable basis for this study. Further analysis for differential gene expression, protein function, enrichment pathways and immune infiltration identified some key factors for pSS. We identified a total of 89 up-regulated genes and 11 down-regulated genes in the dataset. These DEGs were enriched in NOD-like and RIG-I-like receptor signaling pathways, which were significantly associated with the expression of immune cells such as neutrophils, activated dendritic cells, resting NK cells and memory B cells, respectively.

Pattern recognition receptors (PRRs), which include NOD-like receptors (NLRs) and RIG-I like receptors (RLRs), are important sensors for sensing invading viruses (17–19). The primary function of NLRs is to regulate a series of signaling cascades as well as participate in the body's acquired and intrinsic immune responses (20). Members of the NLRs family regulate the development and progression of a variety of diseases, mainly by participating in the inflammatory response or interacting with key proteins of the immune signaling pathway. Important members of the NLRs family include NOD-like receptor pyrin domain-containing protein 1 (NLRP1), NLRP3, NLR family CARD domain-containing protein 4 (NLRC4), neuronal apoptosis inhibitor protein (NAIP), etc. Working as inflammasomes sensors, they induce the secretion of interleukin-1 (IL-1) and IL-18 through binding to their cognate ligands and participate in the immune regulation process of the body. They can create tissue damage and lead to a status of chronic inflammation in some tissues and organs (21–23). In the study by Aigli et al (24), a comparative analysis of gene and protein expression between patients and controls showed that NLRP3 was activated in the infiltrated immune cells of peripheral blood monocytes and saliary glands of patients with pSS. The expression level of NLRP3 is further increased during this process and may affect the severity of the disease. Activation of NLRP3 inflammatory vesicles has been shown to lead to the release of mature IL-1β and IL-18. IL-18 is also significantly elevated in serum and saliva of pSS patients. It is thought to exert a wide range of pro-inflammatory activities and perform a key role in the further expansion of infiltrating damaged tissues of pSS patients (25–27).

The findings of our study revealed that in addition to the NOD-like and RIG-I-like receptor pathways, DEGs were predominantly enriched in infectious diseases, including hepatitis C, measles, influenza A and EBV infections. As an important environmental factor, infection acts as a key player in the initiation and development of pSS (28–30). Innate immunity is the first barrier for the host to block viral infection, and RLRs play a critical role in this process. There are three members of RLRs including retinoic-acid inducible gene I (RIG-I), laboratory of genetics and physiology 2 (LGP2) and melanoma differentiation-associated gene 5 (MDA5) (31). RLRs are cytosolic sensors for virus RNA and the RIG-I-like receptor signaling triggers the secretion of type I interferon and proinflammatory cytokines, which further activate the immune responses (32). Preliminary experiments have been performed to identify the expression patterns of RIG-I and MDA5 in pDCs and monocytes from pSS patients (33). Therefore, the effect of RLRs on pSS is also worth further attention.

Activation of RIG-I and MDA5 triggers the activation of mitochondrial antiviral signaling protein (MAVS), an important junction molecule on downstream mitochondria or peroxisomes, which in turn activates downstream pathways leading to phosphorylation and activation of nuclear factor-κB (NF-κB) and interferon regulating factor 3 (IRF3), eventually resulting in the secretion of type I interferon and proinflammatory factors (34). Interestingly, there is a specific association between NLRs and RLRs in antiviral signaling, actually, NLRs are also involved in the regulation of the type I interferon pathway. Sabbah et al (35) demonstrated that nucleotide-binding oligomerization domain 2 (NOD2) produces an immune response to viral infection and mediates the activation of IRF3, and that this action mediates the direct interaction between NOD2 and MAVS. This reaction occurs when RSV-derived ssRNA is recognized by NOD2 and activates the production of IRF3 and IFN. NLRs have also been demonstrated to negatively regulate the RLR signaling pathway. NLR family member NLRX1 localizes to the outer mitochondrial membrane and interacts with MAVS to inhibit the activation of IRF3 and NF-κB. NLRP5 can also inhibit NF-κB and IFN immune response pathways, possibly by regulating inhibitor of nuclear factor kappa-B kinase (IKK) phosphorylation or by directly binding RIG-I and MDA5 (36–38). In conclusion, NLRs both positively and negatively regulate the RLR signaling pathway.

In addition to immune pathways, immune cell is also the major point of research in pSS. We found that DCs and neutrophils play a more prominent role in pSS. Our findings also suggest a significant association of both DCs and neutrophils with NOD-like and RIG-I-like signaling pathways. DCs not only participate in the body's intrinsic immune response by recognizing and ingesting antigens, but also participate in the adaptive immune response by processing and presenting antigens, as well as regulating the function of other immune cells by secreting chemokines and cytokines. DCs act as important players in the immune process against viral infections mainly through the expression of multiple PRRs, including the NOD-like receptors and RIG-I-like receptors (39). Moreover, it was found that NLRC3 not only reduced the antigen presentation function of DCs, but also decreased the activation and differentiation of CD4+ T cells, resulting in a reduction of Th1 and Th17 subsets, further leading to an inflammatory response(40). With the expression of NOD-like receptors and RIG-like receptors, neutrophils are involved in not only recognizing pathogens but also sensing tissue damage (41). Neutrophils activate NLRP3 inflammasome by releasing danger signals, which can block IL-1β production (42). In addition, neutrophils can also

express both RIG-I and MDA5 receptors and release cytokines as well as alter the expression of genes (43, 44).

In this study, bioinformatic methods were used to identify some possible novel associations between pSS and two receptor signaling pathways, NOD-like and RIG-I-like, thus providing some inspiration for researchers. However, in the absence of experiments, the results of this study have yet to be validated.

Declarations

Acknowledgments

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Conflict of interest

All authors declare they have no conflicts of interest.

References

1. Fox RI. Sjögren's syndrome. The Lancet. 2005;366(9482):321–31. https://doi.org/10.1016/S0140-6736(05)66990-5.
2. Hansena A, Lipskyb PE, Dörner T. Immunopathogenesis of primary Sjögren's syndrome: implications for disease management and therapy. Curr Opin Rheum. 2005;17:558–65. https://doi.org/10.1097/01.bor.0000172801.56744.c3.
3. Kassan SS, Moutsopoulos HM. Clinical manifestations and early diagnosis of Sjögren syndrom. Arch Intern Med. 2004;164:1275–84. https://doi.org/10.1001/archinte.164.12.1275.
4. Ramos-Casals M, Brito-Zeron P, Siso-Almirall A, Bosch X. Primary Sjogren syndrome. BMJ. 2012;344:e3821. https://doi.org/10.1136/bmj.e3821.
5. Qin B, Wang J, Yang Z, Yang M, Ma N, Huang F, et al. Epidemiology of primary Sjogren's syndrome: a systematic review and meta-analysis. Ann Rheum Dis. 2015;74(11):1983–9. https://doi.org/10.1136/annrheumdis-2014-205375.
6. Ekstrom Smedby K, Vajdic CM, Falster M, Engels EA, Martinez-Maza O, Turner J, et al. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph Consortium. Blood. 2008;111(8):4029–38. https://doi.org/10.1182/blood-2007-10-119974.
7. Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjogren syndrome. Nat Rev Rheumatol. 2018;14(3):133–45. https://doi.org/10.1038/nrrheum.2018.1.

8. Stefanski AL, Tomiak C, Pleyer U, Dietrich T, Burmester GR, Dorner T. The Diagnosis and Treatment of Sjogren's Syndrome. Dtsch Arztebl Int. 2017;114(20):354–61. https://doi.org/10.3238/arztebl.2017.0354.
9. Psianou K, Panagoulias I, Papanastasiou AD, de Lastic AL, Rodi M, Spantidea PI, et al. Clinical and immunological parameters of Sjogren's syndrome. Autoimmun Rev. 2018;17(10):1053–64. https://doi.org/10.1016/j.autrev.2018.05.005.
10. Hansen A, Lipsky PE, Dorner T. B cells in Sjogren's syndrome: indications for disturbed selection and differentiation in ectopic lymphoid tissue. Arthritis Res Ther. 2007;9(4):218. https://doi.org/10.1186/ar2210.
11. Hansen A, Reiter K, Ziprian T, Jacobi A, Hoffmann A, Gosemann M, et al. Dysregulation of chemokine receptor expression and function by B cells of patients with primary Sjogren's syndrome. Arthritis Rheum. 2005;52(7):2109–19. https://doi.org/10.1002/art.21129.
12. Nanki T, Takada K, Komano Y, Morio T, Kanegane H, Nakajima A, et al. Chemokine receptor expression and functional effects of chemokines on B cells: implication in the pathogenesis of rheumatoid arthritis. Arthritis Res Ther. 2009;11(5):R149. https://doi.org/10.1186/ar2823.
13. Ramos-Casals M, Font J. Primary Sjogren's syndrome: current and emergent aetiopathogenic concepts. Rheumatology. 2005;44(11):1354–67. https://doi.org/10.1093/rheumatology/keh714.
14. Singh N, Cohen PL. The T cell in Sjogren's syndrome: force majeure, not spectateur. J Autoimmun. 2012;39(3):229–33. https://doi.org/10.1016/j.jaut.2012.05.019.
15. Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjogren's syndrome: what we know and what we should learn. J Autoimmun. 2012;39(1-2):4–8. https://doi.org/10.1016/j.jaut.2012.01.002.
16. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling Tumor Infiltrating Immune Cells with CIBERSORT. Methods Mol Biol. 2018;1711:243–59. https://doi.org/10.1007/978-1-4939-7493-1_12.
17. Barbalat R, Ewald SE, Mouchess ML, Barton GM. Nucleic acid recognition by the innate immune system. Annu Rev Immunol. 2011;29:185–214. https://doi.org/10.1146/annurev-immunol-031210-101340.
18. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11(5):373–84. https://doi.org/10.1038/ni.1863.
19. Rehwinkel J, Reis e Sousa C. RIGorous detection: exposing virus through RNA sensing. Science. 2010;327(5963):284–6. https://doi.org/10.1126/science.1185068.
20. Lupfer C, Kanneganti TD. The expanding role of NLRs in antiviral immunity. Immunol Rev. 2013;255(1):13–24. https://doi.org/10.1111/imr.12089.
21. Anders HJ, Muruve DA. The inflammasomes in kidney disease. J Am Soc Nephrol. 2011;22(6):1007–18. https://doi.org/10.1681/ASN.2010080798.
22. Hayward JA, Mathur A, Ngo C, Man SM. Cytosolic Recognition of Microbes and Pathogens: Inflammasomes in Action. Microbiol Mol Biol Rev. 2018;82(4).

https://doi.org/10.1128/MMBR.00015-18.

23. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med. 2011;17(2):179–88. https://doi.org/10.1038/nm.2279.
24. Vakrakou AG, Boiu S, Ziakas PD, Xingi E, Boleti H, Manoussakis MN. Systemic activation of NLRP3 inflammasome in patients with severe primary Sjögren's syndrome fueled by inflammagenic DNA accumulations. J Autoimmun. 2018;91:23–33. https://doi.org/10.1016/j.jaut.2018.02.010.
25. Bombardieri M, Barone F, Pittoni V, Alessandri C, Conigliaro P, Blades MC, et al. Increased circulating levels and salivary gland expression of interleukin-18 in patients with Sjögren's syndrome: relationship with autoantibody production and lymphoid organization of the periductal inflammatory infiltrate. Arthritis Res Ther. 2004;6(5):R447-56. https://doi.org/10.1186/ar1209.
26. Kleinnijenhuis J, Joosten LA, van de Veerdonk FL, Savage N, van Crevel R, Kullberg BJ, et al. Transcriptional and inflammasome-mediated pathways for the induction of IL-1beta production by Mycobacterium tuberculosis. Eur J Immunol. 2009;39(7):1914–22. https://doi.org/10.1002/eji.200839115.
27. Sakai A, Sugawara Y, Kuroishi T, Sasano T, Sugawara S. Identification of IL-18 and Th17 cells in salivary glands of patients with Sjögren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. J Immunol. 2008;181(4):2898–906. https://doi.org/10.4049/jimmunol.181.4.2898.
28. Croia C, Astorri E, Murray-Brown W, Willis A, Brokstad KA, Sutcliffe N, et al. Implication of Epstein-Barr virus infection in disease-specific autoreactive B cell activation in ectopic lymphoid structures of Sjögren's syndrome. Arthritis rheumatology (Hoboken NJ). 2014;66(9):2545–57. https://doi.org/10.1002/art.38726.
29. Fleck M, Zhang HG, Kern ER, Hsu HC, Müller-Ladner U, Mountz JD. Treatment of chronic sialadenitis in a murine model of Sjögren's syndrome by local fasL gene transfer. Arthritis Rheum.2001;44(4):964–73.https://doi.org/10.1002/1529-0131(200104)44:4<964::AID-ANR154>3.0.CO;2-5
30. Lee SJ, Lee JS, Shin MG, Tanaka Y, Park DJ, Kim TJ, et al. Detection of HTLV-1 in the labial salivary glands of patients with Sjogren's syndrome: a distinct clinical subgroup? J Rheumatol. 2012;39(4):809–15. https://doi.org/10.3899/jrheum.111075.
31. Loo YM, Gale M. Jr. Immune signaling by RIG-I-like receptors. Immunity. 2011;34(5):680–92.
32. Dixit E, Kagan JC. Intracellular pathogen detection by RIG-I-like receptors. Adv Immunol. 2013;117:99–125. https://doi.org/10.1016/j.immuni.2011.05.003.
33. Maria NI, Steenwijk EC, van Helden-Meeuwsen ASIJ, Vogelsang CG, Beumer P. W, et al. Contrasting expression pattern of RNA-sensing receptors TLR7, RIG-I and MDA5 in interferon-positive and interferon-negative patients with primary Sjogren's syndrome. Ann Rheum Dis. 2017;76(4):721–30. https://doi.org/10.1136/annrheumdis-2016-209589.

34. Chan YK, Gack MU. RIG-I-like receptor regulation in virus infection and immunity. Curr Opin Virol. 2015;12:7–14. https://doi.org/10.1016/j.coviro.2015.01.004.
35. Sabbah A, Chang TH, Harnack R, Frohlich V, Tominaga K, Dube PH, et al. Activation of innate immune antiviral responses by Nod2. Nat Immunol. 2009;10(10):1073–80. https://doi.org/10.1038/ni.1782.
36. Benko S, Magalhaes JG, Philpott DJ, Girardin SE. NLRC5 limits the activation of inflammatory pathways. J Immunol. 2010;185(3):1681–91. https://doi.org/10.4049/jimmunol.0903900.
37. Cui J, Zhu L, Xia X, Wang HY, Legras X, Hong J, et al. NLRC5 negatively regulates the NF-kappaB and type I interferon signaling pathways. Cell. 2010;141(3):483–96. https://doi.org/10.1016/j.cell.2010.03.040.
38. Neerincx A, Lautz K, Menning M, Kremmer E, Zigrino P, Hosel M, et al. A role for the human nucleotide-binding domain, leucine-rich repeat-containing family member NLRC5 in antiviral responses. J Biol Chem. 2010;285(34):26223–32. https://doi.org/10.1074/jbc.M110.109736.
39. Fekete T, Agics B, Bencze D, Bene K, Szanto A, Tarr T, et al. Regulation of RLR-Mediated Antiviral Responses of Human Dendritic Cells by mTOR. Front Immunol. 2020;11:572960. https://doi.org/10.3389/fimmu.2020.572960.
40. Fu Y, Zhan X, Wang Y, Jiang X, Liu M, Yang Y, et al. NLRC3 expression in dendritic cells attenuates CD4(+) T cell response and autoimmunity. EMBO J. 2019;38(16):e101397. https://doi.org/10.15252/embj.2018101397.
41. Futosi K, Fodor S, Mocsai A. Neutrophil cell surface receptors and their intracellular signal transduction pathways. Int Immunopharmacol. 2013;17(3):638–50. https://doi.org/10.1016/j.intimp.2013.06.034.
42. Mankan AK, Dau T, Jenne D, Hornung V. The NLRP3/ASC/Caspase-1 axis regulates IL-1beta processing in neutrophils. Eur J Immunol. 2012;42(3):710–5. https://doi.org/10.1002/eji.201141921.
43. Berger M, Hsieh CY, Bakele M, Marcos V, Rieber N, Kormann M, et al. Neutrophils express distinct RNA receptors in a non-canonical way. J Biol Chem. 2012;287(23):19409–17. https://doi.org/10.1074/jbc.M112.353557.
44. Tamassia N, Le Moigne V, Rossato M, Donini M, McCartney S, Calzetti F, et al. Activation of an immunoregulatory and antiviral gene expression program in poly(I:C)-transfected human neutrophils. J Immunol. 2008;181(9):6563–73. https://doi.org/10.4049/jimmunol.181.9.6563.

Figures

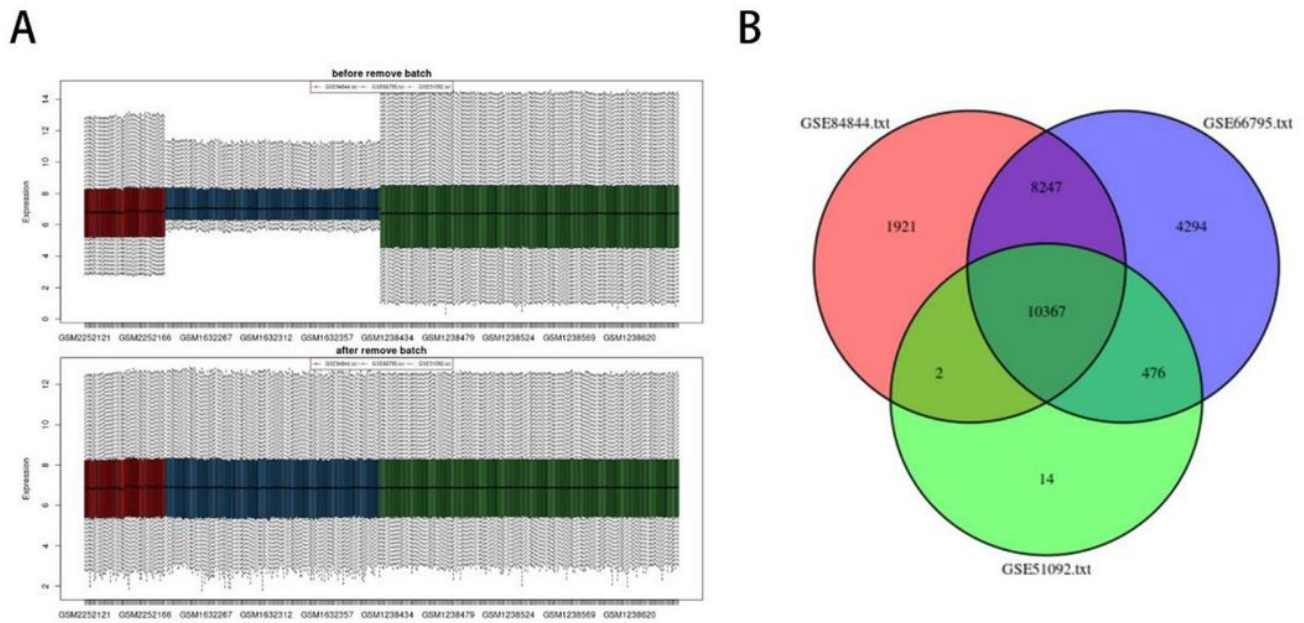

Figure 1

Data integration and removal of batch effects. The Box plots showing the situation of pSS(A) datasets before and after the removal of the batch effect. Venn diagrams showing pSS datasets merged by GSE84844, GSE66795 and GSE51092 (B).

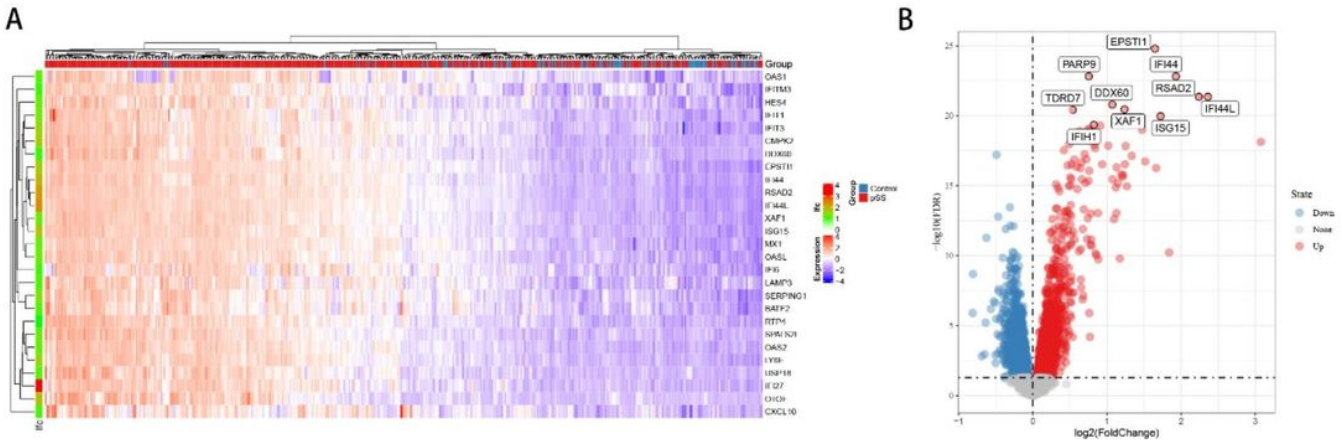

Figure 2

Differentially expressed genes. Heat maps of potentially DEGs between pSS and normal groups (A). Volcano plots showing DEGs in pSS (B). DEGs are those genes with P value <0.05 and |logFC|>0.5. Red indicates relative up-regulated genes and blue indicates down-regulated genes.

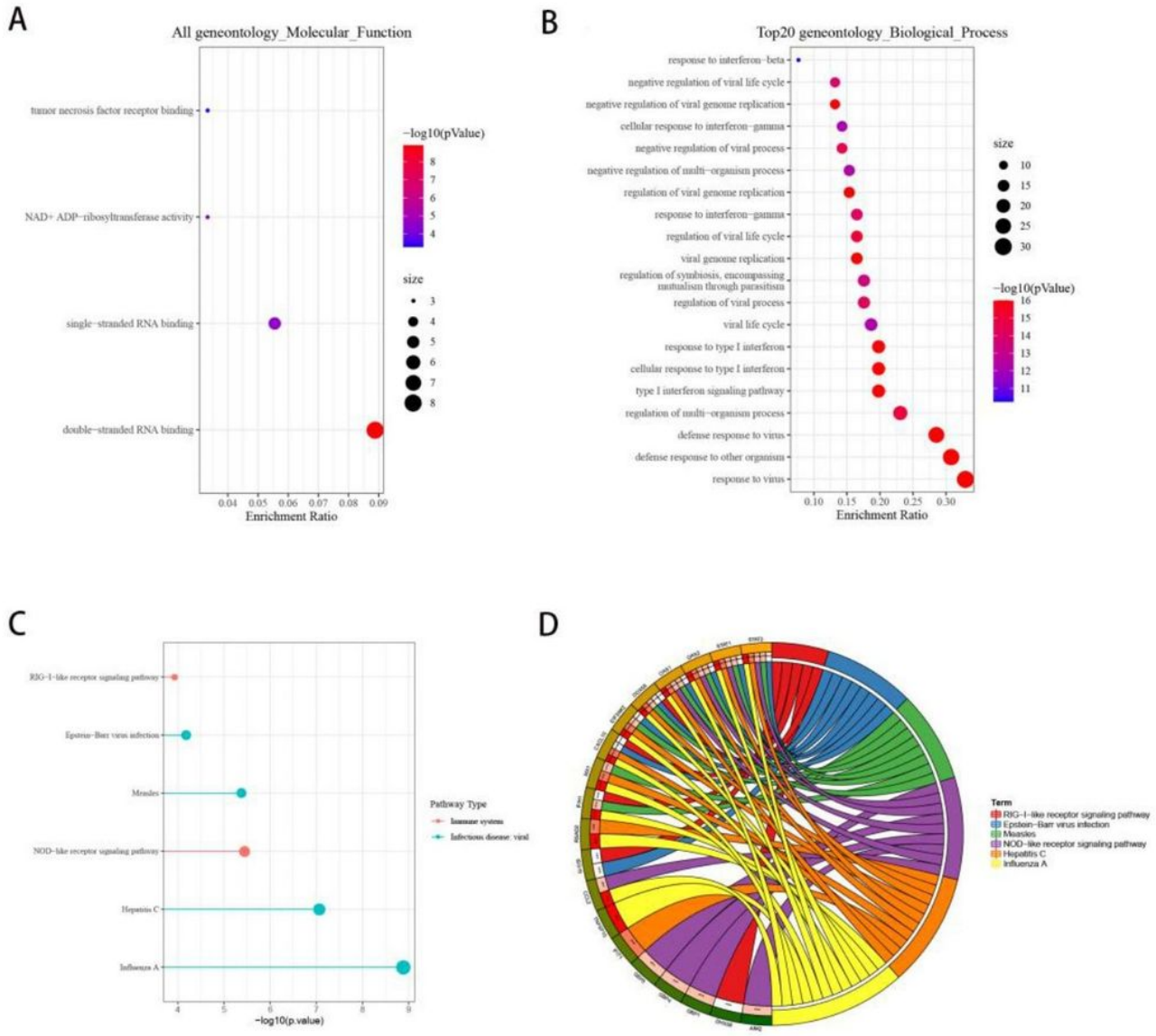

Figure 3

GO and KEGG functional enrichment of DEGs. GO functional enrichment of DEGs (A-B). KEGG enrichment analyses of DEGs (C-D).

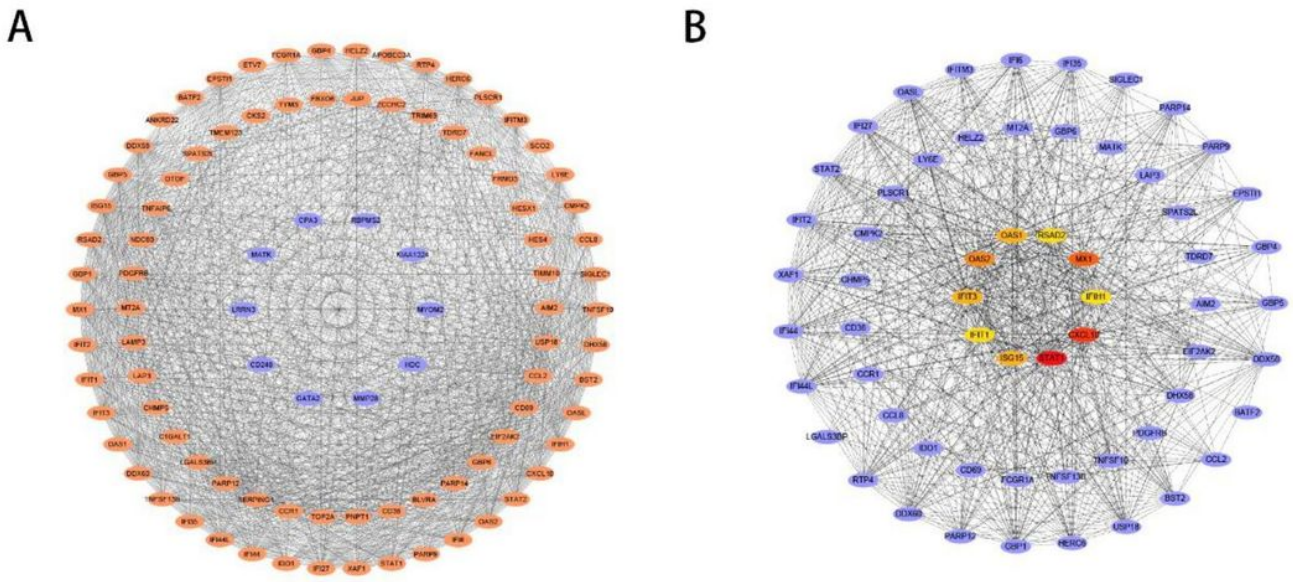

Figure 4

PPI network of dataset(A). Orange represents up-regulated genes and purple represents down-regulated genes. The hub genes were filtered by Cytoscape (B), and the center of the graph shows the top 10 hub genes.

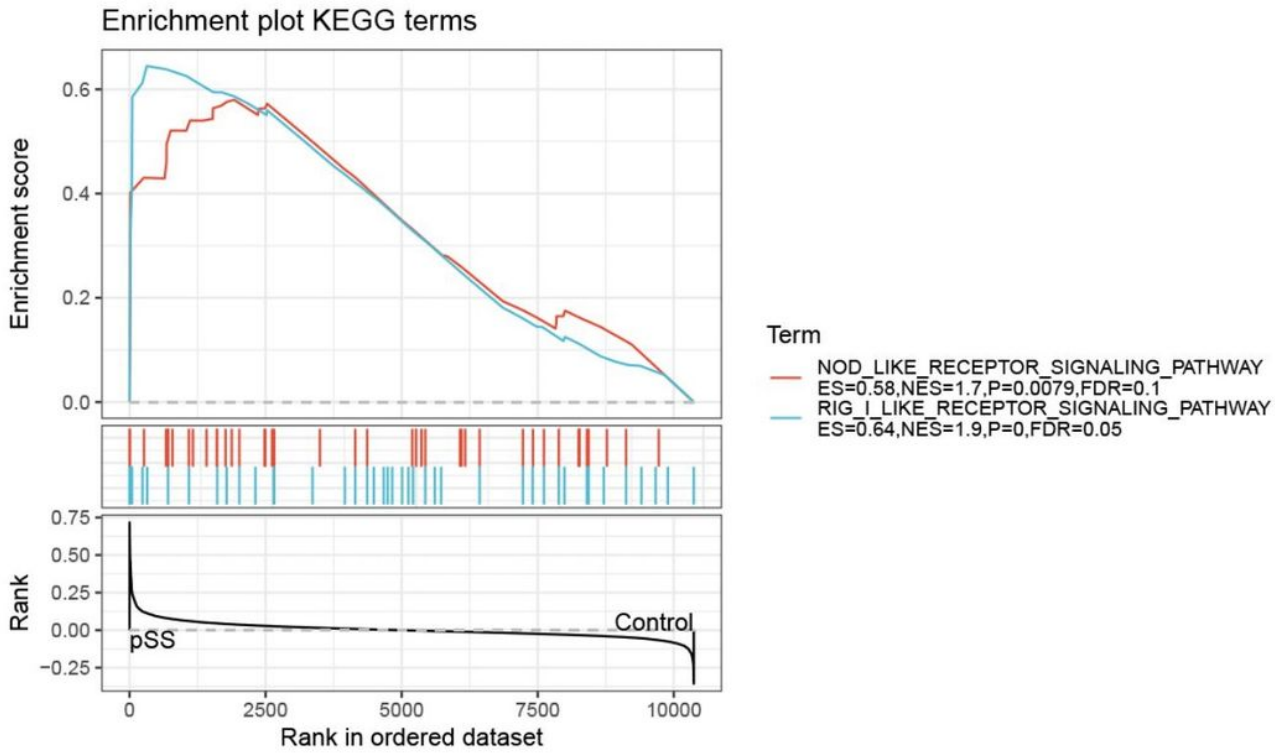

Figure 5

GSEA for significantly enriched in immune system pathways between pSS and control group.

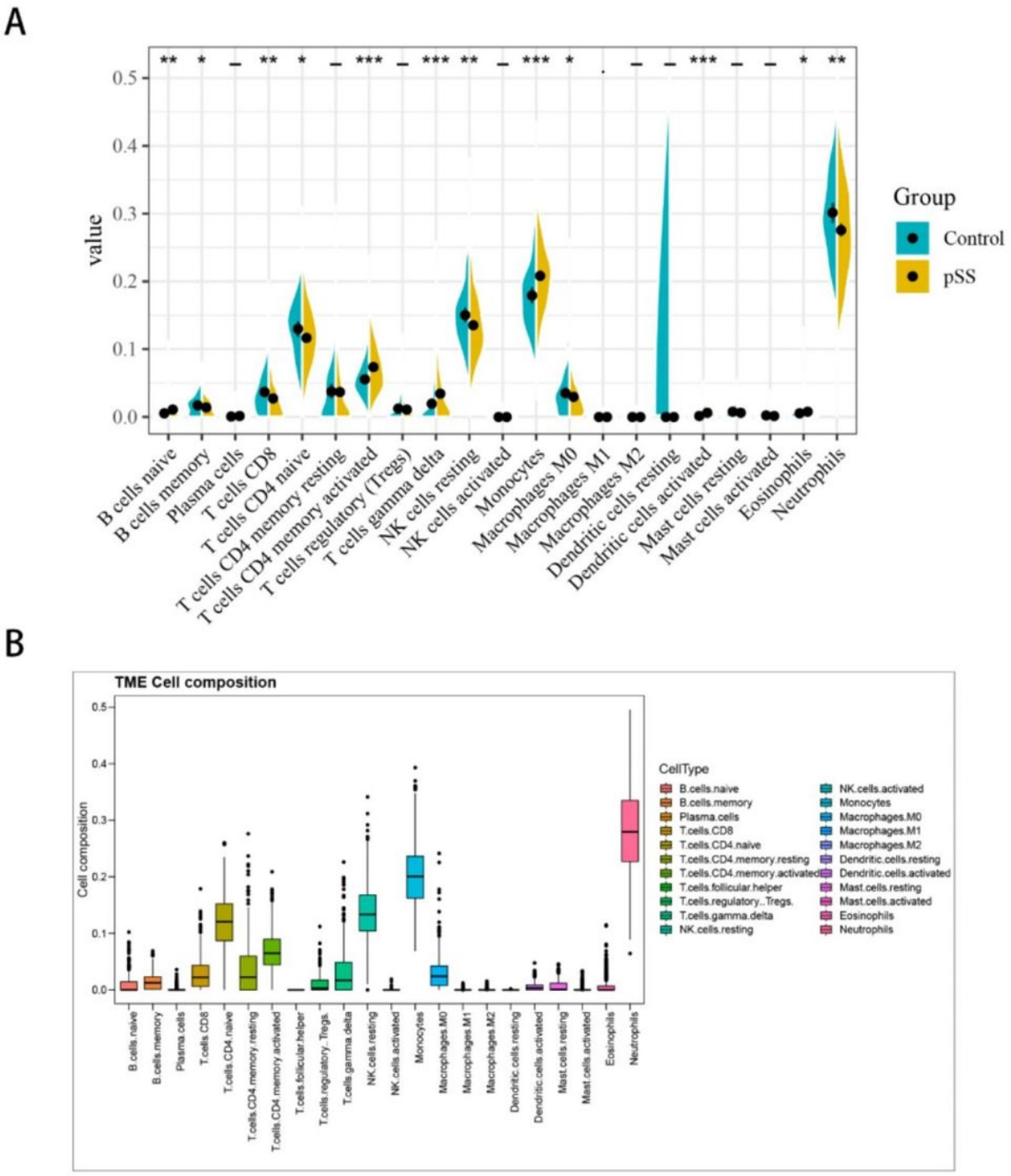

Figure 6

Differential expression of different types of immune cells between pSS and controls. Violin plot shows expression of 22 immune cells in pSS (A). Bar charts shows expression of 22 immune cells in pSS (B).

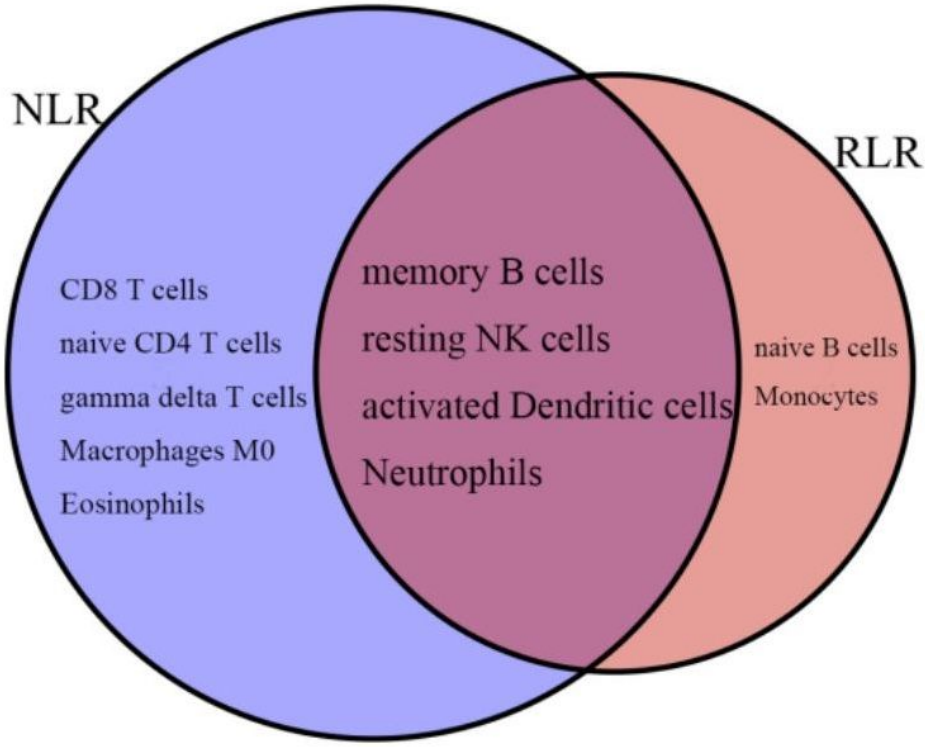

Figure 7

Significantly correlated immune cells in the NOD-like receptor and RIG-I-like receptor signaling Pathway.

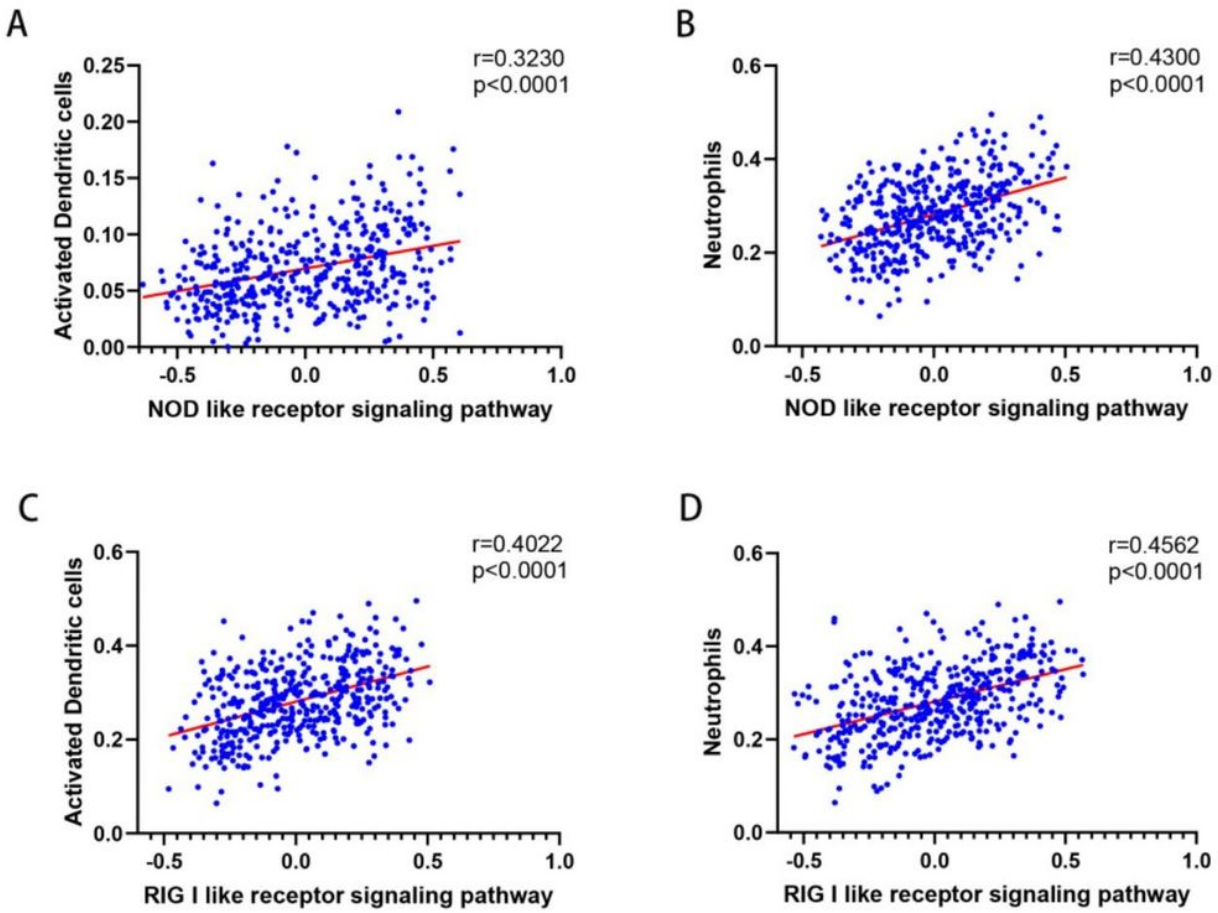

Figure 8

Correlation of the most significantly correlated immune cells, activated DCs and neutrophils with the NOD-like and RIG-I-like receptor signaling Pathway.