



Research article

The critical involvement of monocytes/macrophages in the pathogenesis of primary Sjögren's syndrome: New evidence from Mendelian randomization and single-cell sequencing

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ABSTRACT

Background: Primary Sjögren's syndrome (pSS) stands as a chronic autoimmune disease characterized by an elusive pathogenesis. The synergy of single-cell RNA sequencing and Mendelian randomization (MR) analysis provides an opportunity to comprehensively unravel the contributory role of monocytes/macrophages in the intricate pathogenesis of pSS.

Methods: Differentially expressed genes (DEGs) of various types of immune cells were analyzed after annotating single-cell RNA sequencing (scRNA-seq) data. MR analysis of expression quantitative trait loci (eQTL) and protein quantitative trait loci (pQTL) was conducted to search for key pathogenic genes and proteins. Cellular localization of pathogenic genes was performed based on scRNA-seq data. Variations in signaling pathways between immune cells were further analyzed.

Results: A total of 1434 significant DEGs were identified. Among these, 60 genes exhibited strong relevance to the occurrence of pSS, of which 32 genes differentially expressed in monocytes/macrophages. CTSS was found to be a significant risk protein with a p-value of 0.001 and an odds ratio of 1.384 (1.147–1.669), showing pronounced expression in monocytes/macrophages. Furthermore, monocytes/macrophages displayed heightened expression levels of MXD1, AMPD2, TNFSF10, FTL, UBXN11, CSF3R, and LILRA5. The analysis of intercellular signaling revealed increased signal intensity in both incoming and outgoing signals in monocytes/macrophages. The signaling interactions between monocytes/macrophages, B cells, and T cells exhibited varying degrees of deviation.

Conclusions: This study highlights the significant involvement of monocytes/macrophages in the pathogenesis of pSS, as evidenced by MR analysis and scRNA-seq analysis. This suggests monocytes/macrophages as a focal point for pathogenesis research and potential therapeutic targeting in pSS.

1. Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disorder characterized by the dysfunction and inflammation of exocrine glands, particularly the salivary and lacrimal glands. It is recognized as one of the most prevalent autoimmune rheumatic

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diseases. Population-based studies reported an annual incidence of pSS ranging from 3.90 to 11.77 per 100,000 inhabitants, with higher rates observed in Asia [1]. While pSS can manifest at any age, it mainly emerges in individuals aged 40 and above, with a prevalence approximately ten times higher in females than males. Remarkably, around 13 % of pSS patients experience potentially severe complications affecting non-glandular organ systems, such as interstitial lung disease, myelitis, thrombocytopenia, and lymphoma, which may contribute to an elevated mortality risk [2].

The therapeutic management pSS has undergone limited substantive changes in recent decades. The availability of disease-modifying treatment options, particularly for glandular manifestations, remains scarce [3]. A prominent contributing factor could be the incomplete understanding of its pathogenesis. The pathological features of pSS can be classified as either germinal-centre-like structures around epithelial tissues of parenchymal organs or deposition of immunocomplexes caused by excessive activation of B cells [4]. Thus, the activation of B cells stands out as a pivotal element in the pathogenesis of pSS. However, pSS should not be perceived as a solely B-cell-driven disorder. An array of compelling evidence indicated the participation of other immune cell types, encompassing monocytes/macrophages, diverse subsets of T cells, natural killer (NK) cells [5,6]. Various cells intricately intertwine to form an immune response network, contributing to the pathogenesis of pSS.

Previous research tended to center around predetermined cell types and lacked a comprehensive outlook on the disruption of immune network balance. In this study, Mendelian randomization (MR) analyses of expression quantitative trait loci (eQTL) and protein quantitative trait loci (pQTL) were employed to elucidate the key pathogenic genes and proteins of pSS, and the expression patterns of these genes across immune cells were delineated utilizing single-cell RNA sequencing (scRNA-seq) data. Integrated with the analyses of alterations in intercellular signals among immune cells, the study explored the possible crucial pathogenic cells in the progression of pSS. Leveraging a novel analytical approach, this research offered a fresh perspective on the pathogenesis of pSS, thereby contributing to the identification of new therapeutic targets.

2. Methods

2.1. Analysis of single-cell RNA sequencing

The scRNA-seq data of peripheral blood mononuclear cells (PBMCs) from 5 patients with pSS and 5 healthy controls (HC) were retrieved from GEO database under accession GSE157278 (<https://www.ncbi.nlm.nih.gov/geo/>) [7]. Quality control, analysis, and exploration of the scRNA-seq data were performed using the R package "Seurat" (<https://github.com/satijalab/seurat>) [8]. The specific process was as follows: 1) The red blood cells were first removed. 2) The unqualified cells were screened according to cell features (nFeature_RNA >200, nCount_RNA <30000 and percent.mt < 30). 3) Data normalization was executed using the package "SCTransform", which provided functions for batch correction and data correction [9]. Although a relatively clear separation was observed between pSS patients and HC, there was no significant batch effect within the pSS patients. Similarly, there was no significant separation within the HC group (Additional file 1 Fig. S1). Therefore, the separation between pSS and HC might tend to be due to biological effects. Clusters of cells were determined through a shared nearest neighbor (SNN) modularity optimization-based clustering algorithm via the "FindClusters" function, employing a resolution of 0.6. The identification of differentially expressed genes was conducted using the "FindAllMarkers" function, with a logFC threshold of 0.25 and min pct of 0.25.

2.2. Cell annotation and differentially expressed genes (DEGs) based on cell types

Cell annotation primarily relied upon published literature and the CellMarker database (<http://bio-bigdata.hrbmu.edu.cn/CellMarker/>). T cells were identified by expression of CD3D and CD3E. NK cells were characterized by the expression of NKG7, GNLY and CD247. The recognition of B cells were based on CD79A, CD79B, CD37. Monocytes and macrophages exhibited expression characteristics of CD14, CD68, S100A12, LYZ. The recognition genes of NKT cells were NKTR. Dendritic cells (DCs) were distinguished by CST3 and CD1C, while plasmacytoid dendritic cells were identified by LILRA4 and JCHAIN. Mast cell were recognized by GATA2, CPA3 [10]. After cell annotation, differentially expressed genes were analyzed for each cell type with the function "FindAllMarkers". Genes with adjusted P value < 0.05 and an average log2FC > 0.3 were considered significant DEGs and proceeded to the next stage of analysis.

2.3. Mendelian randomization analysis of eQTLs and pQTLs

The eQTLs utilized in the MR analysis were selected based on significant DEGs and were derived from eQTLGen database (<https://eqtlgen.org>). The pQTLs for the MR analysis were selected based on significant eQTLs and were sourced from deCODE database (<https://www.decode.com/>) [11]. The genome-wide association (GWAS) data of pSS was acquired from IEU OpenGWAS project (<https://gwas.mrcieu.ac.uk/>) and the original data came from the FinnGen study [12]. The criteria for the selection of instrumental variables (IVs) consisted of the following: (1)The IV was associated with the risk factor. (2) The IV was not associated with confounders. (3) The IV influenced outcome only through the risk factor.

In cases where only one single nucleotide polymorphism (SNP) was available for constructing the instrumental variable, the result was determined using the Wald ratio. In scenarios involving multiple SNPs, the primary result was obtained from the inverse-variance weighted regression model (IVW). The selection of IV was based on the threshold of $p < 5 \times 10^{-8}$ and $r = 0.001$ [13]. Cochran's Q statistic were performed to assess the level of heterogeneity of each SNP. Detection of horizontal pleiotropy in causal relationships inferred from the MR analysis was conducted using the MR-PRESSO test and MR Egger test. A P value < 0.05 for the main result was

considered statistically significant. Leave-one-out analysis was executed to exhibit the individual effects of each SNP on the overall results and demonstrate the robustness of the results. R package “TwoSampleMR” (<https://mrcieu.github.io/TwoSampleMR/>) and “MR-PRESSO” (<https://github.com/rondolab/MR-PRESSO>) were applied for MR analysis [14,15]. The scatter plot visualized the casual effect of pQTL on pSS risk. The forest plot depicted the impact of each SNP in the pQTL analysis, along with the overall effect.

2.4. Cell-cell interaction analysis

Cell-cell interaction analysis was conducted by R package “Cellchat” (<https://github.com/sqjin/CellChat>) [16]. The integrated ligand-receptor interaction database, ChatDB, amalgamates information regarding signaling molecule interactions from the KEGG Pathway database as well as experimental investigations.

Deconvolution of the complex intercellular communications in signaling network using centrality measures from network were used to determine major signaling sources networks across datasets.

3. Results

3.1. Analysis and identification of peripheral blood mononuclear cells

Substandard cells were selectively excluded after quality control, resulting in the analysis of 28289 cells from five healthy controls and 31086 cells from five patients with pSS. A total of 30 clusters were identified, mainly divided into B cells, dendritic cells, mast cells, megakaryocytes, monocytes/macrophages, NK cells, plasmacytoid dendritic cells and T cells. The aggregation of distinct cell types was depicted in Fig. 1A using umap, while Fig. 1B and Additional file 2 Fig. S2 showcased the categorization of these cells based on whether they originated from healthy controls or pSS patients. The expression of characteristic genes (based on the average logFC) in each cluster were performed in Fig. 1C, in which the phenotype of each cell type can be clearly seen. Fig. 1D illustrated the relative proportions of diverse cell types in patients with pSS as compared to HC.

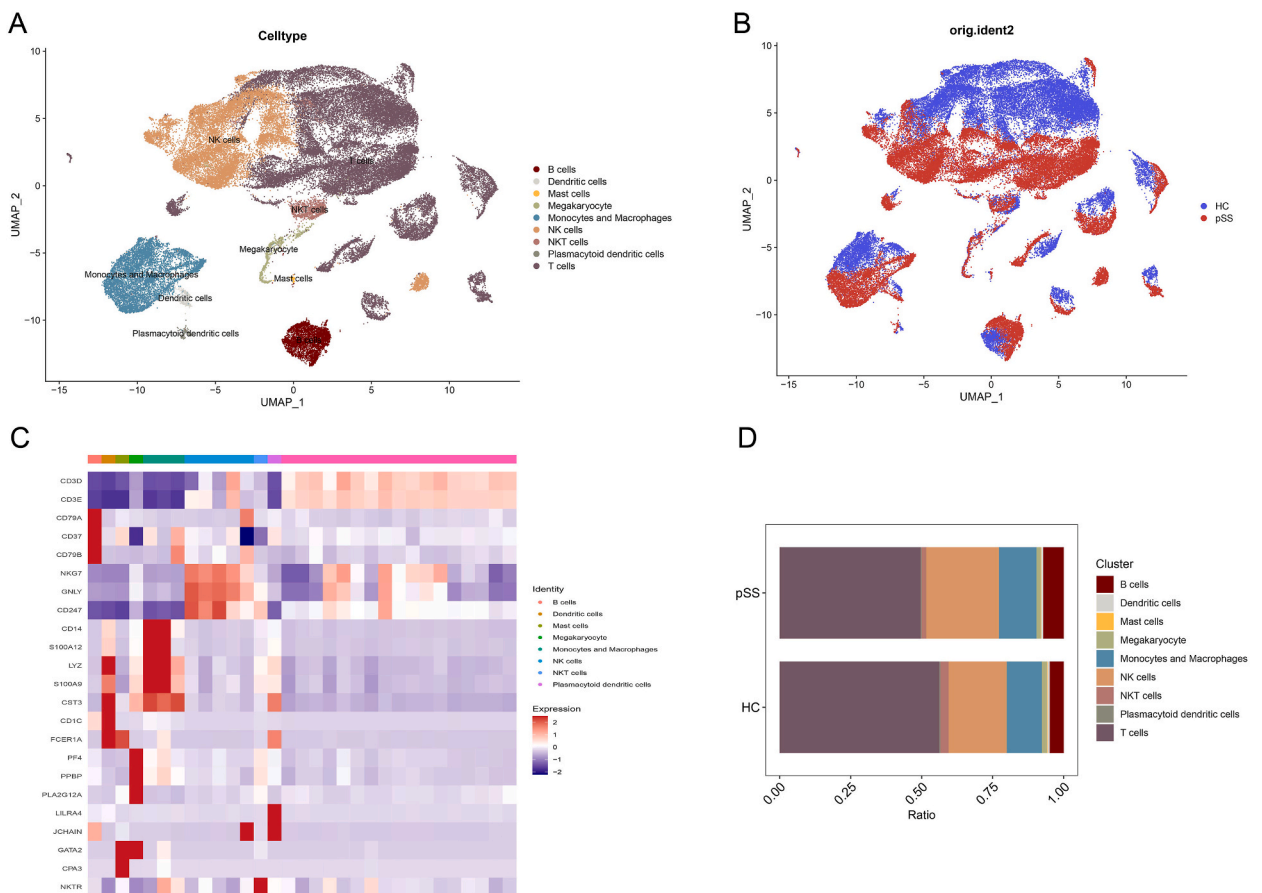


Fig. 1. Clustering and annotation of single cells (A) Cell type annotation for different cell clusters (B) Cell clustering of pSS patients and HC (C) Expression of characteristic molecules in different cell types (D) Cell ratio of pSS patients and HC.

3.2. Differentially expressed genes (DEGs) based on cell types

Genes with adjusted P value < 0.05 and average log2FC > 0.3 were designated as significant DEGs. A total of 785 significant DEGs were ascertained in monocytes/macrophages, compared to 473 in B cells, 254 in dendritic cells, 34 in megakaryocytes, 577 in NK cells, 231 in NKT cells, and 610 in T cells. Among the 785 genes exhibiting significant differential expression in monocytes/macrophages, 440 were up-regulated and 345 were down-regulated. More detailed information about the significant DEGs of each cell type could be found in the Additional file 3 Table S1. Mast cells and plasmacytoid dendritic cells were precluded in this analysis due to the insufficient cell count. Fig. 2 visualized the significant DEGs across each cell type, accompanied by labels for the top five up-regulated and down-regulated genes.

3.3. Mendelian randomization analysis of eQTLs

There were 1434 significant DEGs after the elimination of duplicates, of which 1318 eQTLs without repetitions were matched. These eQTLs were taken as exposure, with pSS serving as the outcome, and subsequent MR analysis was performed separately on each eQTL. Wald ratio was adopted as main result for single SNP, IVW was adopted as the main result. The main result P value < 0.05 was considered significant. These outcomes underwent further scrutiny to assess the fulfillment of the three hypotheses. Two eQTLs associated with genes NELL2 and KLRC3 were subjected to recalibration, involving the removal of IVs that did not meet the criteria of hypothesis three. After verification by MR-PRESSO and MR-EGGER, it is found that eQTL related to CSF3R performed significant heterogeneity. Therefore, the main source of heterogeneity, rs3917922, was removed. The re-calculated outcome remained statistically significant, while heterogeneity was successfully mitigated. A total of 60 genes were ultimately identified to be significant related to the occurrence of pSS. Among these genes, 25 exhibited odds ratios below 1, indicating a protective effect, while 35 displayed odds ratios above 1, signifying risk factors. Of these 60 genes, 32 were significantly differentially expressed in monocytes/macrophages, suggesting the profound involvement of monocytes/macrophages in the pathogenesis of pSS.

However, whether genes were risk or protective does not precisely coincide with their up-regulated or down-regulated states. On one hand, of the 35 risk genes, 23 exhibited up-regulation, while the remaining 12 displayed down-regulation. On the other hand, of the 25 protective genes, 14 were down-regulated and 11 up-regulated. This outcome highlights that while up-regulated risk-associated genes and down-regulated protective genes constitute the predominant trend, instances of reverse patterns also emerge, thus emphasizing the intricate and multifaceted roles played by these genes in the pathogenesis of pSS.

Among the 35 risk genes, 10 were up-regulated in monocytes/macrophages, while 8 were down-regulated. Simultaneously, among the 25 protective genes, 9 were down-regulated in monocytes/macrophages, while 5 were up-regulated. This indicated that the involvement of monocytes/macrophages in the pathogenesis of pSS was also complicated. Detailed information of MR analysis were performed in Table 1. Fig. 3 illustrated the causal effect of pathogenic genes in monocytes/macrophages.

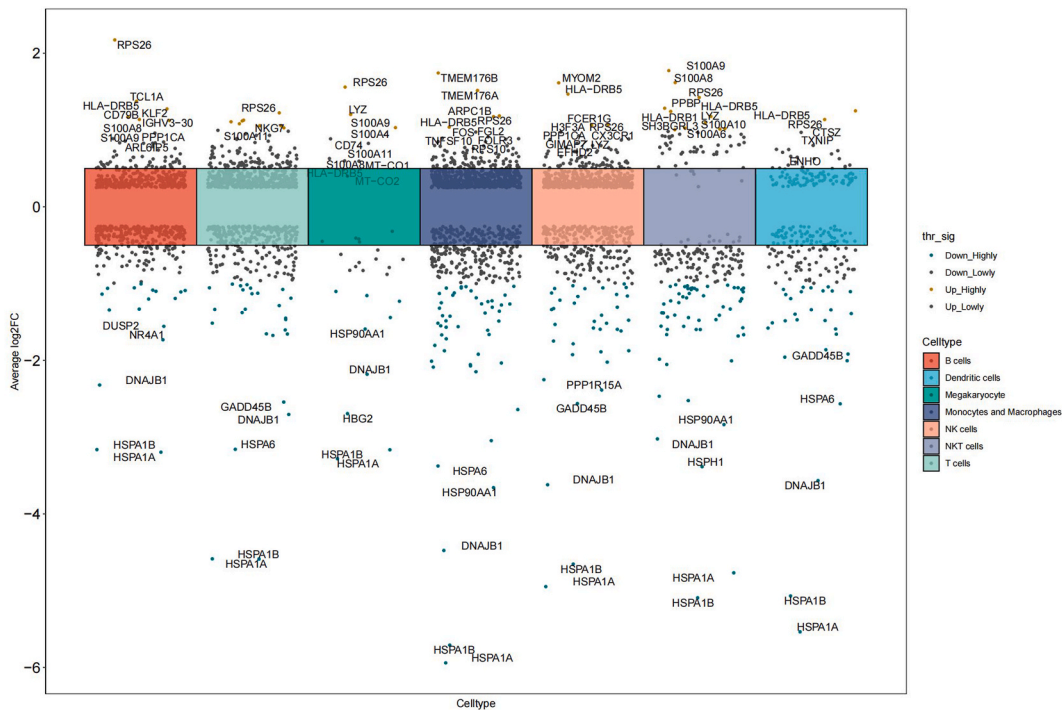


Fig. 2. Differentially expressed genes in different immune cells.

3.4. Mendelian randomization analysis of pQTLs

Sixty genes related to the occurrence of pSS were previously been identified. The pQTLs related to these genes were further validated by MR analysis to explore whether the proteins expressed by these genes were key pathogenic proteins. A total of 31 pQTLs were matched in the DECODE database. Among these proteins, CTSS emerged as a significant risk factor for the development of pSS, with a P-value of 0.001 and an OR of 1.384(1.147–1.669) according to the result of IVW. More detailed information, along with

Table 1
Mendelian randomization analysis of significant eQTLs.

gene	method	b	se	OR	P-value	MR-PRESSO	Cochran's Q	MR-Egger
JARID2	IVW	-0.271	0.089	0.763	0.002	0.606	0.406	0.609
IL32	IVW	0.203	0.084	1.225	0.015	0.520	0.318	0.566
SEC62	Wald ratio	-0.547	0.235	0.579	0.020	/	/	/
MXD1	IVW	-0.462	0.227	0.630	0.042	/	0.673	0.855
MEF2C	IVW	0.213	0.096	1.237	0.027	0.588	0.553	0.392
RPS5	Wald ratio	0.190	0.093	1.209	0.040	/	/	/
FTL	Wald ratio	0.894	0.452	2.444	0.048	/	/	/
DYNLL1	Wald ratio	1.129	0.488	3.092	0.021	/	/	/
DHRS7	IVW	0.254	0.086	1.289	0.003	0.646	0.395	0.672
CHMP4B	IVW	-0.162	0.077	0.850	0.036	0.893	0.833	0.677
UBE2I	IVW	-0.264	0.098	0.768	0.007	/	0.655	/
STX10	IVW	0.683	0.252	1.981	0.007	0.245	0.114	0.859
TBCB	IVW	-0.231	0.099	0.794	0.020	/	0.817	/
HSPB1	Wald ratio	0.820	0.395	2.270	0.038	/	/	/
PTGDS	IVW	0.310	0.130	1.363	0.017	/	0.529	0.510
BACH2	IVW	-0.453	0.160	0.636	0.005	0.758	0.619	0.918
ATP6V0E1	Wald ratio	0.775	0.380	2.171	0.041	/	/	/
AMPD2	Wald ratio	1.081	0.405	2.946	0.008	/	/	/
UAP1	Wald ratio	0.333	0.163	1.395	0.041	/	/	/
APH1A	Wald ratio	-1.171	0.497	0.310	0.018	/	/	/
CSF3R	IVW	-0.952	0.434	0.386	0.022	0.051	0.060	0.140
IRF2BPL	IVW	0.169	0.084	1.184	0.043	0.501	0.591	0.311
DUSP1	Wald ratio	-0.872	0.331	0.418	0.009	/	/	/
TNFSF10	IVW	-0.383	0.148	0.682	0.010	0.523	0.308	0.878
ZBTB1	Wald ratio	1.721	0.513	5.592	0.001	/	/	/
SDF2L1	IVW	0.677	0.265	1.967	0.011	/	0.632	/
SKIL	IVW	-0.735	0.343	0.479	0.032	/	0.672	/
RPS3A	IVW	0.568	0.269	1.765	0.035	/	0.756	/
NFKBIE	IVW	-0.208	0.101	0.812	0.039	0.534	0.560	0.291
DYNLT1	IVW	-0.411	0.164	0.663	0.012	/	0.841	/
NR4A2	Wald ratio	-1.011	0.316	0.364	0.001	/	/	/
CHD1	Wald ratio	0.944	0.470	2.570	0.045	/	/	/
AZIN1	IVW	0.196	0.094	1.216	0.037	0.820	0.947	0.615
UBXN11	IVW	0.542	0.250	1.719	0.030	/	0.451	/
SPON2	IVW	0.303	0.112	1.354	0.007	0.516	0.534	0.699
G6PD	Wald ratio	1.314	0.492	3.720	0.008	/	/	/
ITGB2	IVW	0.153	0.064	1.166	0.016	0.703	0.774	0.785
RPL8	IVW	0.141	0.058	1.151	0.014	/	0.343	/
CYB561A3	Wald ratio	1.019	0.443	2.769	0.021	/	/	/
IER5	IVW	-0.257	0.097	0.774	0.008	/	0.643	0.779
CTSS	IVW	0.203	0.074	1.225	0.006	0.798	0.775	0.270
KRTCAP2	Wald ratio	-1.211	0.412	0.298	0.003	/	/	/
TPRA1	Wald ratio	0.214	0.095	1.239	0.025	/	/	/
UQCRQ	Wald ratio	0.882	0.445	2.416	0.047	/	/	/
CITED2	IVW	-0.267	0.129	0.766	0.039	/	0.715	0.612
S1PR1	Wald ratio	-0.847	0.286	0.429	0.003	/	/	/
PGAM1	IVW	0.320	0.123	1.376	0.010	/	/	/
RGS19	Wald ratio	1.188	0.445	3.281	0.008	/	/	/
NOP10	IVW	0.137	0.069	1.147	0.047	0.402	0.427	0.865
IST1	Wald ratio	-0.954	0.278	0.385	0.001	/	/	/
UQCR10	Wald ratio	1.163	0.296	3.201	0.000	/	/	/
NELL2	IVW	-0.178	0.090	0.837	0.011	0.648	0.841	0.109
FMNL1	Wald ratio	0.908	0.417	2.480	0.029	/	/	/
LILRA5	IVW	-0.225	0.092	0.798	0.014	0.677	0.322	0.590
SEMA4D	IVW	-0.205	0.080	0.814	0.010	0.752	0.704	0.512
HACD4	IVW	0.361	0.184	1.434	0.050	0.331	0.135	0.186
HLA-C	IVW	0.337	0.114	1.401	0.021	0.171	0.002	0.343
KLRC3	IVW	0.154	0.062	1.167	0.013	0.961	0.967	0.923
HLA-DPA1	IVW	-0.382	0.110	0.683	0.000	0.433	0.086	0.922
HLA-DQA1	Wald ratio	-0.532	0.096	0.587	0.000	/	/	/

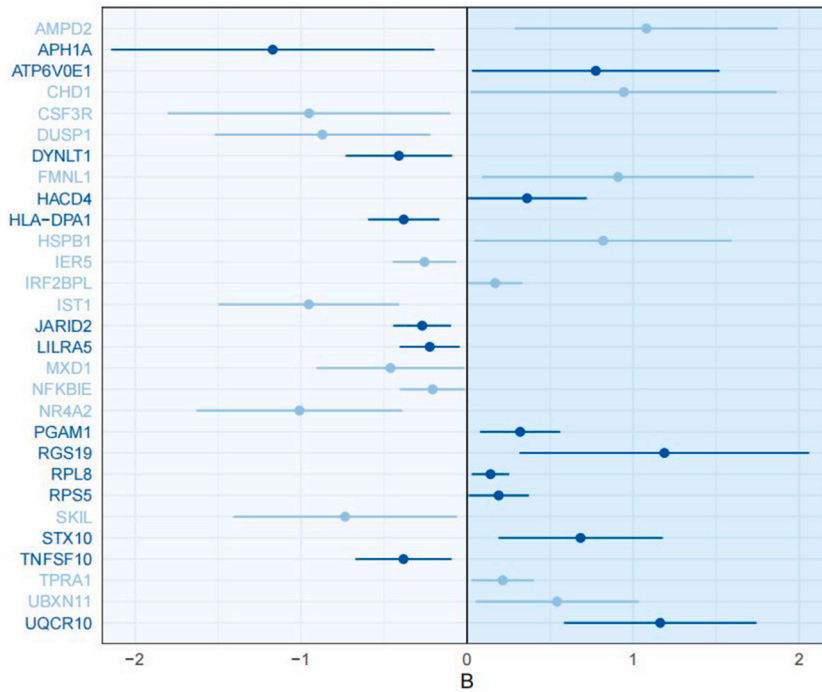


Fig. 3. The causal effect of genes significantly related to the occurrence of pSS in monocytes/macrophages (dark blue: up-regulated genes in monocytes/macrophages, light blue: genes down-regulated in monocytes/macrophages).

outcomes from four other methodologies, was presented in Table 2. Twenty-four SNPs that fit the hypothesis were selected as IV during the analysis, all possessing F-values exceeding 10. Detailed information were performed in the Additional file 3 Table S2. The P-value of MR-PRESSO was 0.48 and the P-value of MR-egger intercept test was 0.70, indicating no heterogeneity. The P-value of Cochran’s Q test was 0.37, indicating no pleiotropy. Fig. 4A performed the casual effect of CTSS on pSS risk. Fig. 4B showed the effect of each SNP and also the total effect. Through the leave-one-out analysis, it was ascertained that no single SNP exerted undue influence on the overall outcome, thus confirming the robustness of the result. The funnel plot manifested that the two sides were basically symmetrical, indicating a lack of significant bias on either side.

3.5. The expression of key genes in immune cells

The expression of 60 key genes related to the occurrence of pSS was shown in Fig. 5 and Additional file 4 Fig. S3. MXD1, AMPD2, TNFSF10, FTL, UBXN11, CTSS, CSF3R and LILRA5 exhibited heightened expression levels within monocytes/macrophages compared to other cell types (Fig. 5A–H). Notably, the expression of CTSS, CSF3R and LILRA5 in monocytes/macrophages was relatively specific, with CTSS having been previously identified as a key pathogenic protein in the earlier analysis. Moreover, MEF2C displayed elevated expression in both monocytes/macrophages and B cells (Fig. 5I). The distinct expression pattern of these pivotal genes within monocytes/macrophages further accentuated the potential pivotal role of monocytes/macrophages in the complicated pathogenesis of pSS.

3.6. Overall analysis of intercellular signaling

The total incoming and outgoing signal strengths for each cell type were presented in Fig. 6A and B. The findings revealed noticeable signal alterations in monocytes/macrophages, T cells, B cells, and NK cells. Among them, monocytes/macrophages

Table 2
Mendelian randomization analysis of CTSS.

Method	b	se	pval	or
MR Egger	0.283	0.145	0.064	1.327(0.999–1.764)
Weighted median	0.347	0.121	0.004 ^a	1.415(1.116–1.796)
Inverse variance weighted	0.325	0.096	0.001 ^a	1.384(1.147–1.669)
Simple mode	0.215	0.273	0.438	1.240(1.147–1.669)
Weighted mode	0.341	0.115	0.007 ^a	1.407(1.123–1.763)

^a P < 0.05.

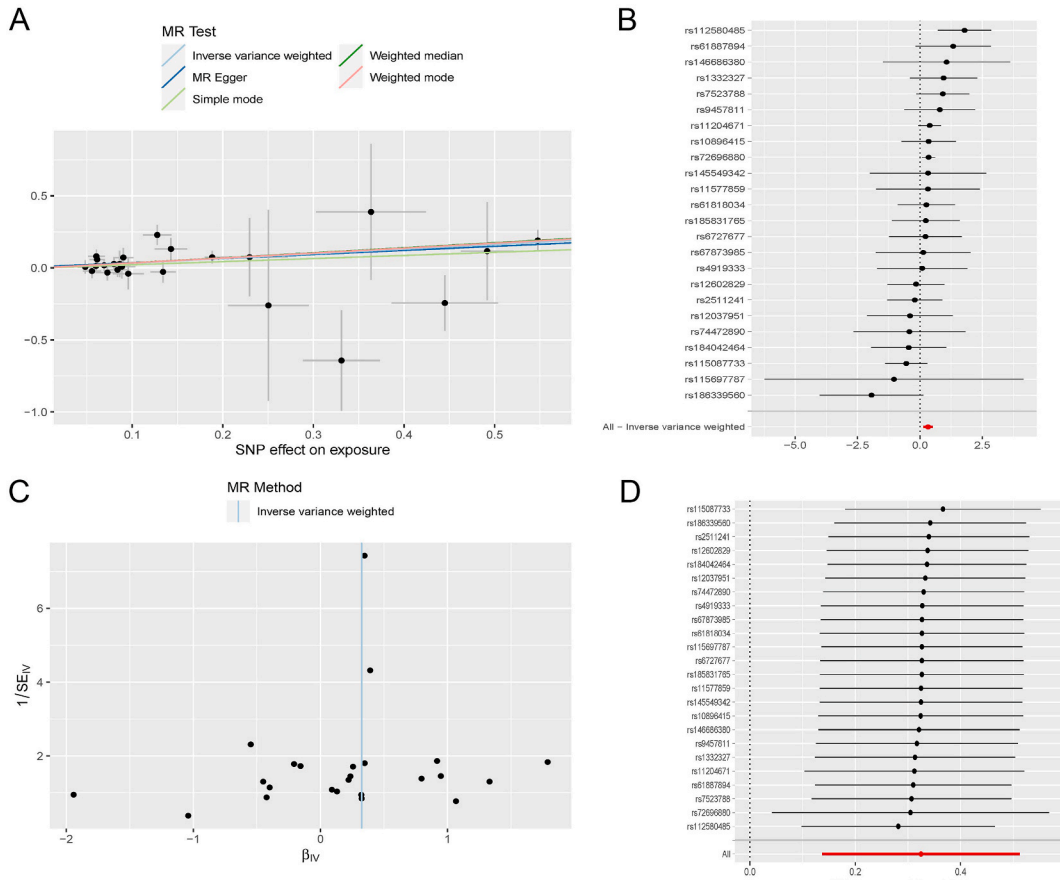


Fig. 4. Mendelian randomization analysis of CTSS (A) Scatterplot of the effect sizes (B) Forrester plot of the effect size of each SNP (C) Funnel plot (D) Leave-one-out analysis.

exhibited apparent enhancement in both incoming signal and outgoing signals. T cells displayed enhanced incoming signal intensity, while B cells demonstrated an increased outgoing signal. In contrast, NK cells exhibited a decrease in incoming signal intensity alongside an elevated outgoing signal.

Fig. 6C and D depicted the major intercellular signal pathways in HC and pSS patients. The relative ranking of “CD99”, “ADGRE5”, “CD48”, “LCK”, “PCAM1” and “BAFF” in the signaling pathway increased, while the ranking of “MHC-II”, “APP”, “ICAM”, “THBS”, “SEMA4”, “COLLAGEN” and “VISFATIN” decreased. “TNF”, “CD86”, and “IFN-II”, observed in HC, were absent in pSS patients due to inadequate signal strength, while “BLTA” was exclusively observed in pSS patients.

3.7. Monocytes/macrophages-associated intercellular signaling pathways

The main outgoing signal pathways of monocytes/macrophages were “MHC-I”, “ADGRE5”, “ICAM”, “RESISTIN”, “BAFF” and “VISFATIN”, while the main incoming signal pathways of monocytes/macrophages were “GALECTIN”, “COLLAGEN” and “SEMA4”. Meanwhile, “ANNEXIN” and “PECAM1” exhibited both strong signal inputs and outputs. Fig. 7 displayed the monocytes/macrophages-associated intercellular signaling pathways between HC and pSS patients.

3.8. Changes in monocytes/macrophages-related intercellular signaling pathways

This section provided a detailed analysis of pathways that have undergone noteworthy and significant alterations. According to the signal pattern, these pathways could be divided into three types. The first were the pathways that mainly displayed the outgoing signals, including “ADGRE5”, “ICAM”, “RESISTIN” and “BAFF”. The second were the pathways characterized by incoming signals, such as “GALECTIN” and “SEMA4”. The last were the pathways with both high outgoing and high incoming signals, such as “ANNEXIN” and “PECAM1”.

The results showed enhanced communication between monocytes/macrophages and other immune cells in pSS patients, including increased intercellular interaction and enhanced signaling strength. In pSS patients, monocytes/macrophages sent more signals to T cells (“RESISTIN” and “GALECTIN”), and received more signals from T cells (“ADGRE5”, “ICAM” and “ANNEXIN”). Similarly, signals

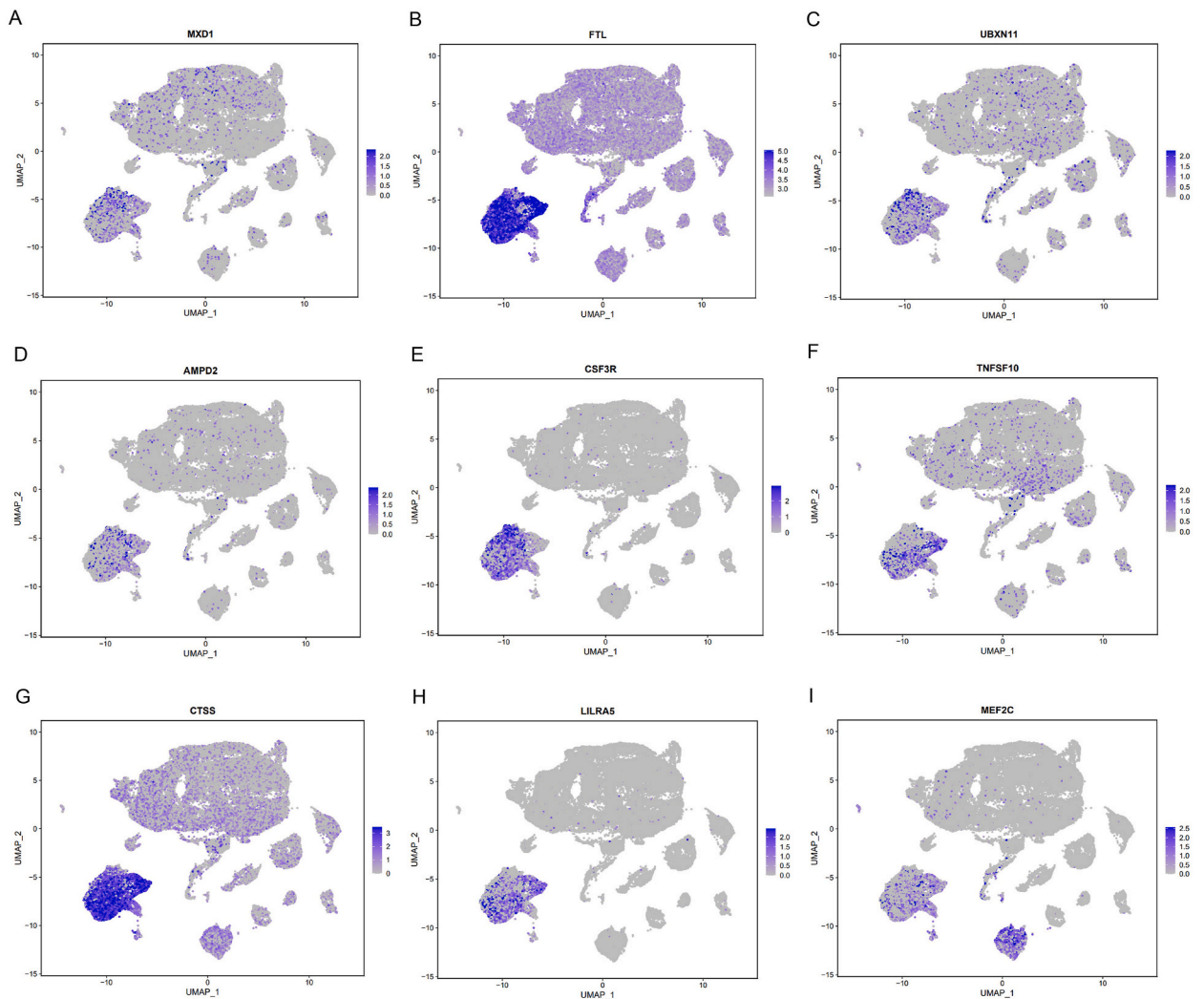


Fig. 5. The expression of key genes in different cell types (A) MXD1 (B) FTL (C) UBXN11 (D) AMPD2 (E) CSF3R (F) TNFSF10 (G) CTSS (H) LILRA5 (I) MEF2C.

sent to B cells (“ADGRE5”, “RESISTIN”, “BAFF” and “GALECTIN”) and received from B cells (“ICAM”) were both enhanced. Likewise, the intercellular interaction between monocytes/macrophages and NK cells also increased (“ADGRE5”, “RESISTIN”, “GALECTIN” and “ANNEXIN”). In addition, several pathways showed enhanced signaling to monocytes/macrophages themselves (“ADGRE5”, “RESISTIN”, “GALECTIN”, “ANNEXIN” and “PECAM1”).

Table 3 summarized the specific changes of these signaling pathways in three aspects: intercellular interaction, signal strength, and the roles in the signal networks. Descriptions such as “New” or “Absent” or “Heightened” or “Attenuated” in the table were based on the comparison of pSS patients with healthy controls. Table 3 also provided links to figures that depicted the changes for each signaling pathway.

4. Discussion

This study provided a comprehensive perspective that highlighted the critical role of monocytes/macrophages in the pathogenesis of pSS, a facet previously not well-addressed. The results furnished a twofold substantiation of this notion, encompassing both the expression of pathogenic genes and proteins within monocytes/macrophages, as well as the signal fluctuations of monocytes/macrophages within the immune cell network.

Macrophages display remarkable vigor in the realm of innate immunity while they also contribute to the initiation of adaptive immunity by recruiting various immune cells, including lymphocytes. Macrophages has been classified as M1 macrophages, which promote inflammation or as alternatively M2 macrophages, which mitigate inflammation and facilitate tissue repair [17].

The pathological progression of pSS may, in part, be attributed to tissue-resident macrophages. Analyses revealed a conspicuous

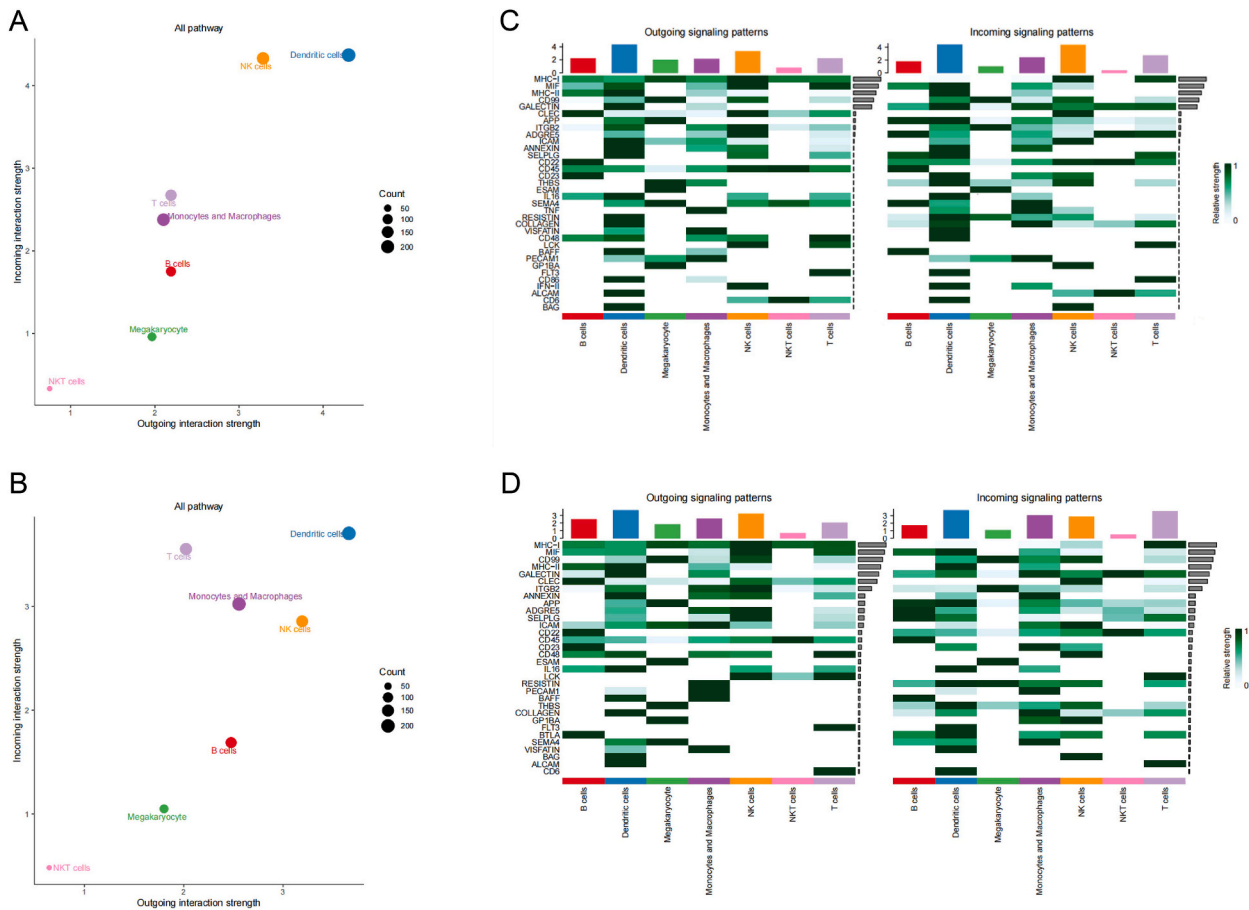


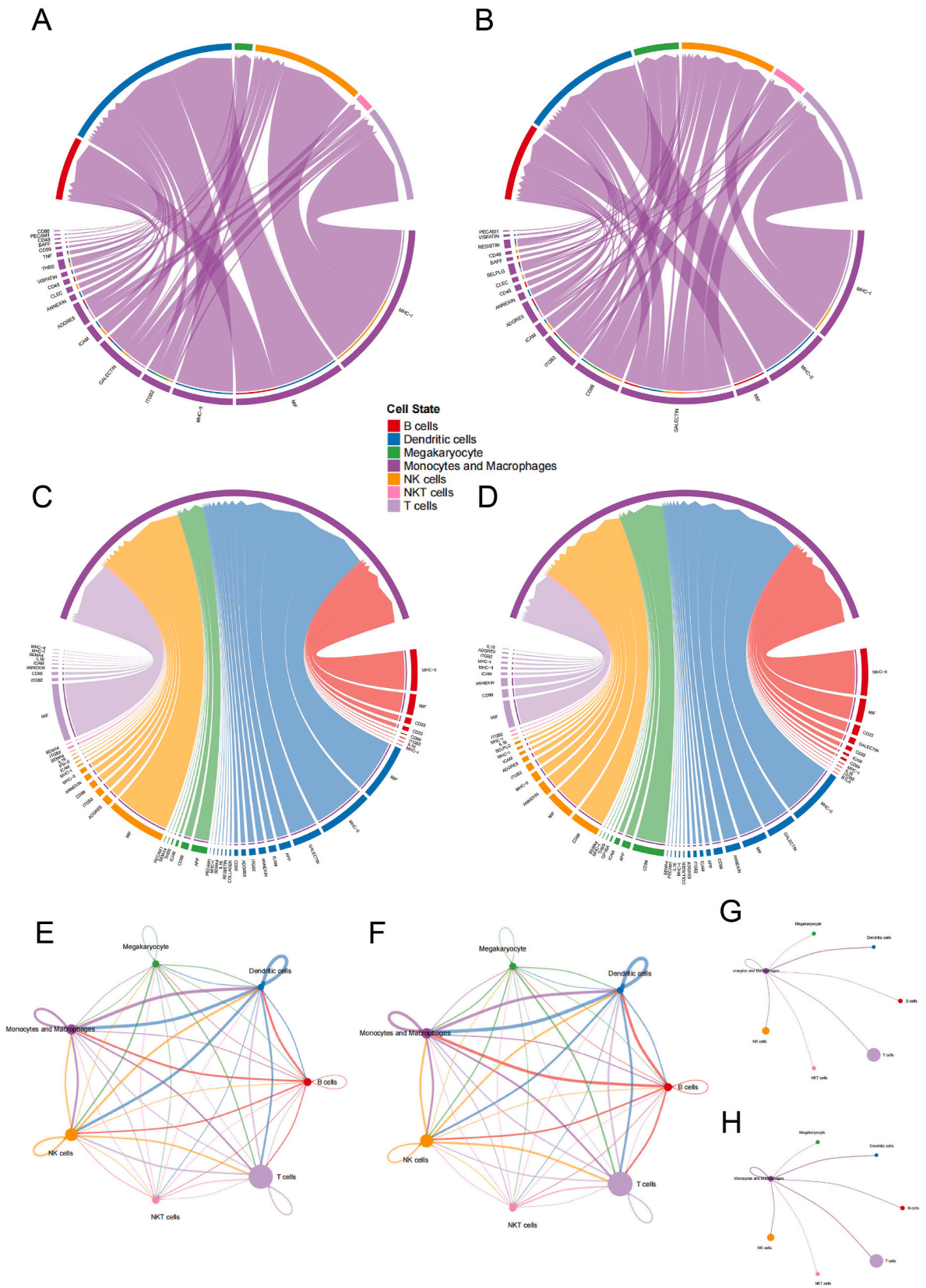
Fig. 6. Global intercellular signaling between HC and pSS patients (A) Overall incoming and outgoing signal strength in HC (B) Strength of intercellular signaling pathway in HC (C) Overall incoming and outgoing signal strength in pSS patients (D) Strength of intercellular signaling pathway in pSS patients.

abundance of M1 macrophage infiltration in pSS patients compared to normal human salivary gland tissue [18]. A more specific analysis showed that the presence of macrophages in the labial salivary glands in pSS patients was positively associated with biopsy focus score, parenchymal-organ involvement, lymphoma and cryoglobulinemia [19]. The process of salivary glands damage can be partially explained by the secretion of IL-18, nitric oxide and metalloproteases by macrophages [20–22]. Beyond the salivary glands, macrophages can exert similar effects on other epithelial tissues such as the ocular epithelium [23]. In addition, several studies have suggested that both tissue-resident and circulating macrophages are involved in the development of lymphoma in pSS patients [19,20,24], which also indicating an essential interplay between macrophages and lymphocytes in pSS.

CTSS, a cysteine endopeptidase belonging to the papain family, is notably abundant within lysosomes. CTSS is highly expressed primarily in antigen-presenting cells (APCs) and is involved in major histocompatibility complex class II (MHC-II) mediated immune responses, implying that inhibition of CTSS could be a promising approach in attenuating autoantigen-specific CD4⁺T cells responses [25]. Overexpression of CTSS in lacrimal gland was considered to be associated with the ocular manifestations of pSS and therapeutic interventions targeting CTSS have demonstrated efficacy in non-clinical studies of pSS [26–28]. Our findings indicated the significance of CTSS as a key risk gene in pSS, while the relatively specific expression of CTSS in monocytes/macrophages suggested that monocytes/macrophages portrayed exceptional significance in the MHC-II mediated immune response in pSS.

The results of intercellular signals suggested an intensification of interactions between monocytes/macrophages and other immune cells, especially B cells and T cells, in pSS patients when compared to HC.

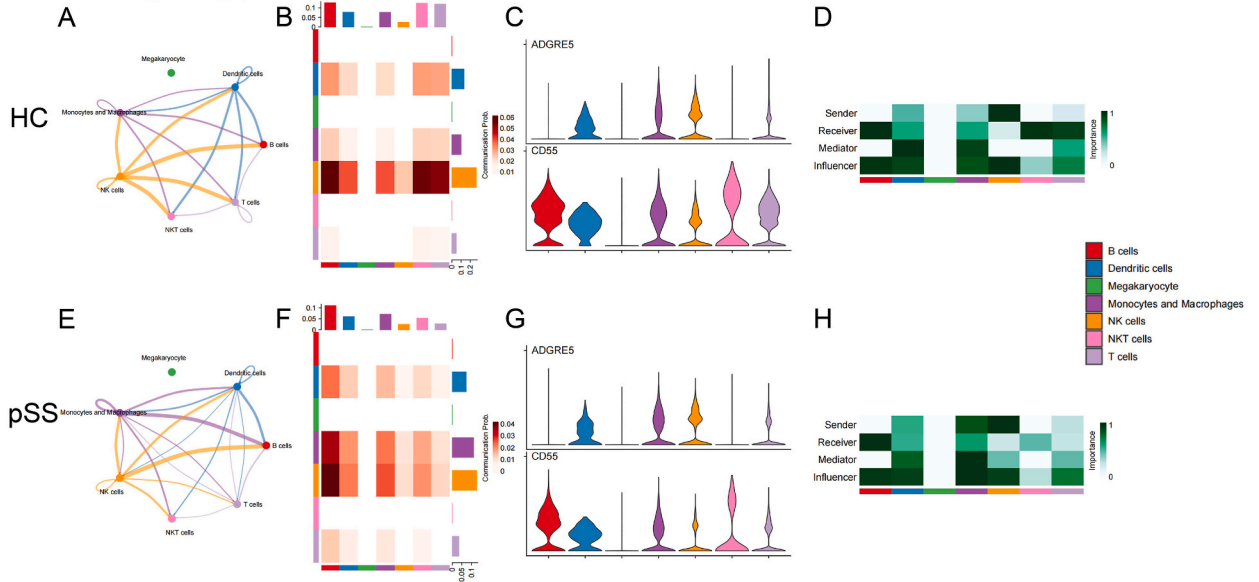
The obvious changes in the communication between monocytes/macrophages and B cells in pSS patients are noteworthy, given the widely acknowledged role of B cells as effector cells in pSS pathogenesis [29]. The BAFF signaling pathway, chiefly composed of BAFF and BAFFR, stands as a pivotal signal governing the survival and maturation of B cells [30]. Elevated BAFF levels have been reported in pSS patients, correlating with heightened lymphocytic infiltration that may partly contribute to ectopic germinal center formation [31]. The encouraging outcomes from BAFF receptor blockade treatment trials hold promise as a potential breakthrough in the current therapeutic landscape, albeit requiring further clinical data for validation [32]. The intensified BAFF signaling pathway between monocytes/macrophages and B cells observed in the study could imply an increased capacity of monocytes/macrophages to stimulate



(caption on next page)

Fig. 7. Monocytes/macrophages-associated intercellular signaling pathways (A) Pathways send from monocytes/macrophages in HC (B) Pathways send from monocytes/macrophages in pSS patients (C) Pathways send to monocytes/macrophages in HC (D) Pathways send to monocytes/macrophages in pSS patients (E) Signal interaction between immune cells in HC (F) Signal interaction between immune cells in patients with pSS (G) Signal intensity that monocytes/macrophages send to other immune cells in HC (H) Signal intensity that monocytes/macrophages send to other immune cells in patients with pSS.

ADGRE5 signaling pathway



ICAM signaling pathway

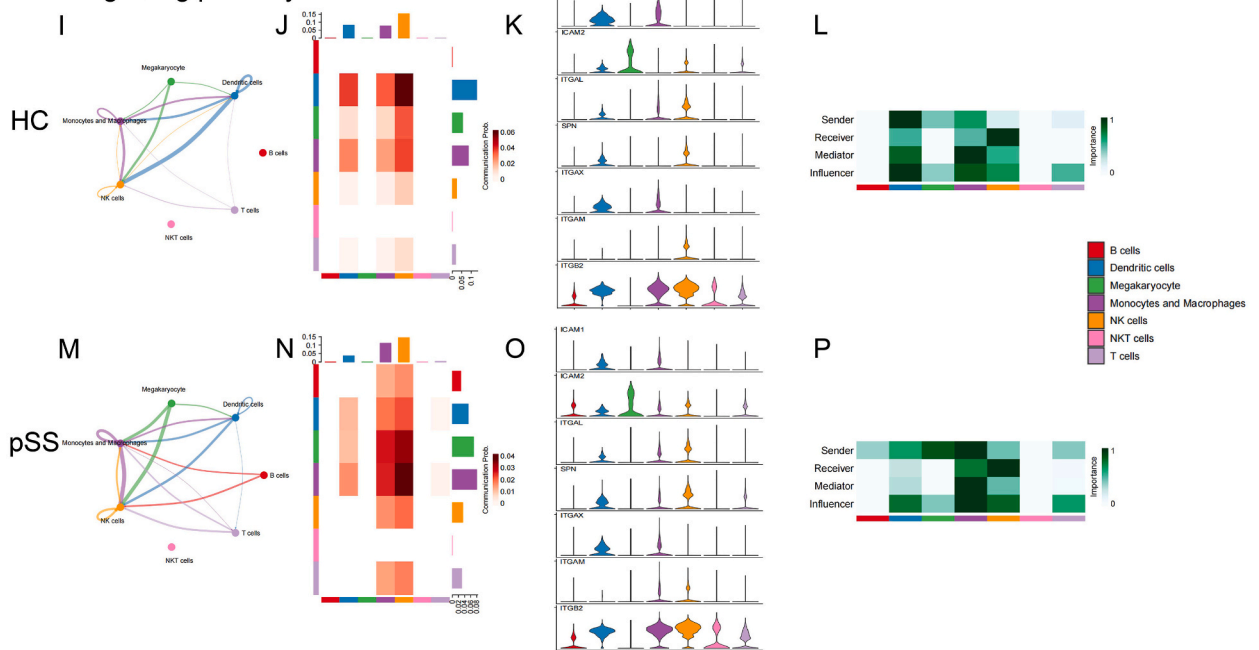


Figure 8-1. The intercellular signal strength, the intensity of the signals sent and received by each cell type, the expression of receptors and ligands on the cell surface, and the role of each cell type in the signaling network of the main outgoing signal pathways in monocytes/macrophages between HC and pSS patients (A)-(H) ADGRE5 signaling pathway (I)-(P) ICAM signaling pathway.

RESISTIN signaling pathway

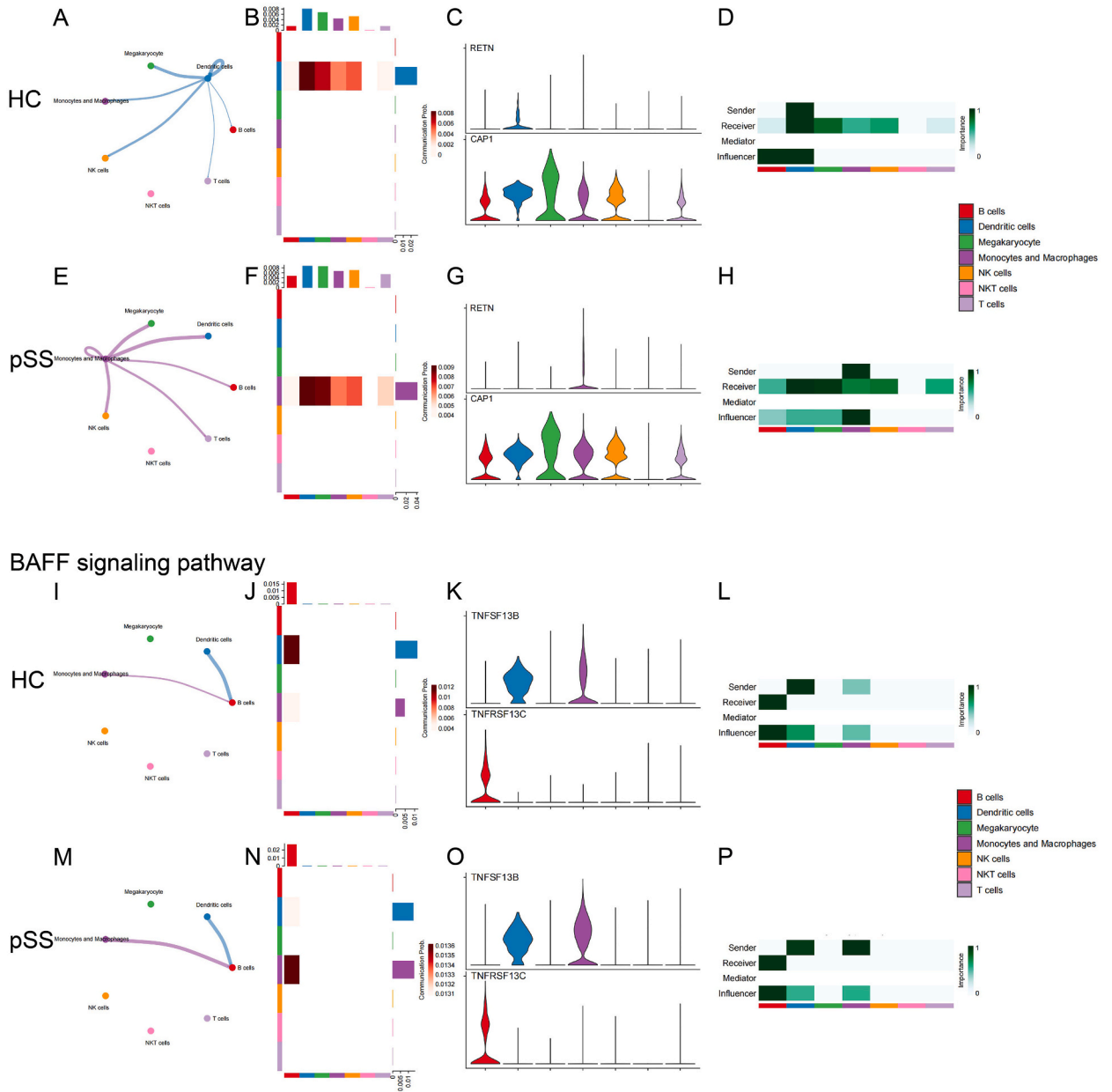
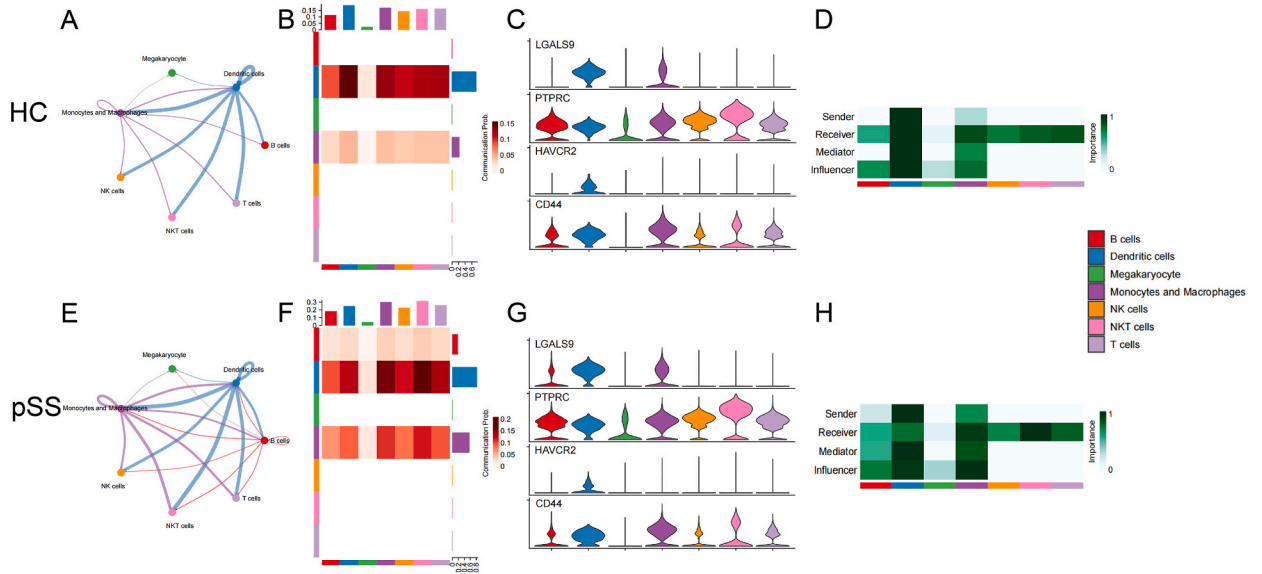


Figure 8-2. The intercellular signal strength, the intensity of the signals sent and received by each cell type, the expression of receptors and ligands on the cell surface, and the role of each cell type in the signaling network of the main outgoing signal pathways in monocytes/macrophages between HC and pSS patients (A)-(H) RESISTIN signaling pathway (I)-(P) BAFF signaling pathway.

B cells. Contrastingly, the enhanced GALECTIN signaling pathway suggested an inhibition of B cell triggered by monocytes/macrophages, as galectin-9, binding with CD45, suppressed calcium signaling via a Lyn-CD22-SHP-1 dependent mechanism, ultimately dampening B cell activation [33]. Another notable observation was that B cells expressed more ICAM2 and monocytes/macrophages expressed more ITGAM in pSS patients, culminating in the reinforcement of ICAM2-(ITGAM + ITGB2) and ICAM2-(ITGAL + ITGB2) pathways, thereby facilitating cell adhesion [34,35]. The observed increased ADGRE5-CD55 signaling also supported enhanced adhesion to supporting cells [36].

Similarly, enhanced cell adhesion was observed between monocytes/macrophages and T cells in pSS patients, mainly evident through increased ADGRE5 and ICAM signaling. In addition, a noteworthy alteration was the reduction in SEMA4D expression, not only observed in T cells but also in NK cells. It functioned in T cell priming [37] and was able to drive CD8⁺ T-Cell lesional trafficking in oral lichen planus via CXCL9/CXCL10 up-regulations [38].

GALECTIN signaling pathway



SEMA4 signaling pathway

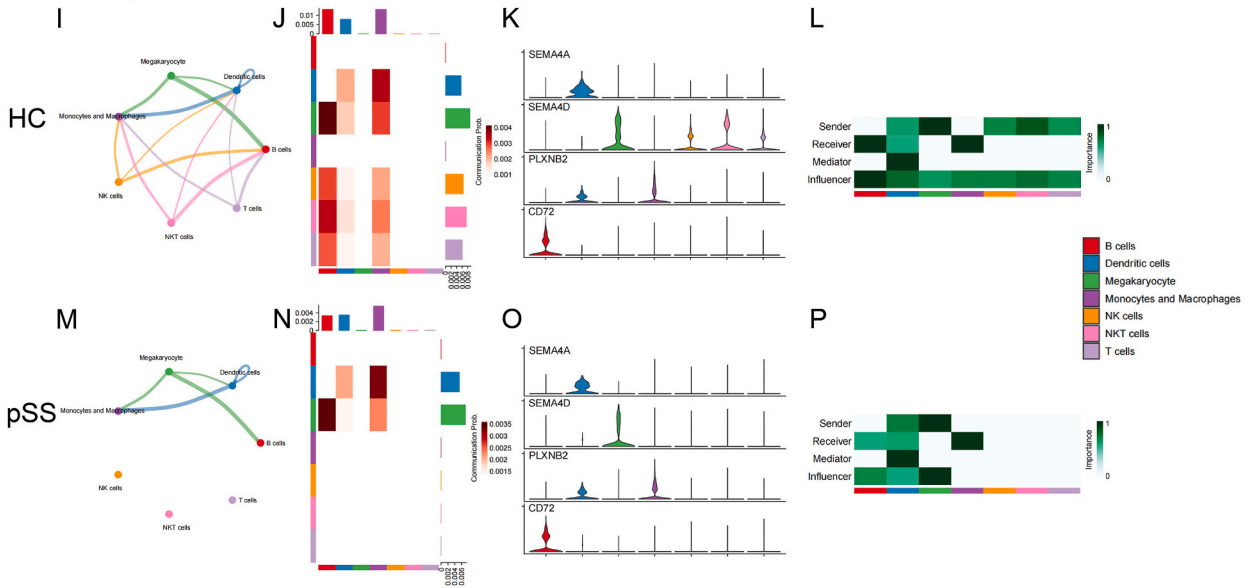
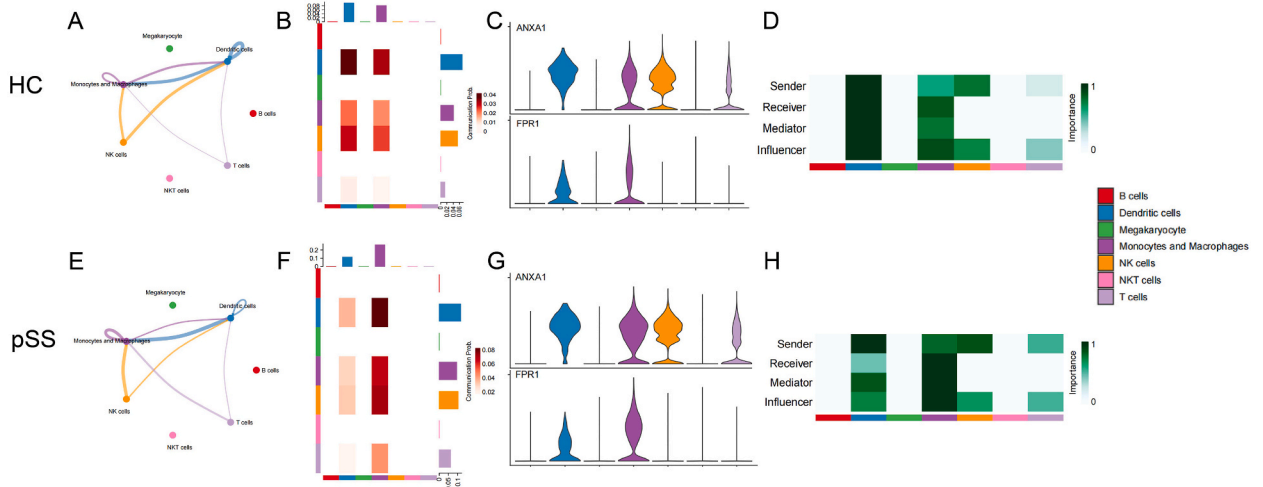


Fig. 9. The intercellular signal strength, the intensity of the signals sent and received by each cell type, the expression of receptors and ligands on the cell surface, and the role of each cell type in the signaling network of the main incoming signal pathways in monocytes/macrophages between HC and pSS patients (A)–(H) GALECTIN signaling pathway (I)–(P) SEMA4 signaling pathway.

Another crucial aspect is the self-regulation of monocytes/macrophages. While RETN was primarily expressed on DCs in HC, scRNA-seq data revealed a remarkably shift, with demonstrating monocytes/macrophages as the main source of RETN expression in pSS patients. Meanwhile, the up-regulation of resistin was observed during monocyte/macrophage differentiation, shedding light on resistin’s role in monocyte/macrophage function [39,40]. Monocytes/macrophages also exhibited a significant enhancement of cell-adhesion-related pathways, including not only the previously discussed ADGRE5 and ICAM signaling pathways, but also ANXA1-FPR1 signaling, given that FPR1 participated in a signal relay which regulated the infiltration of phagocytes to inflammatory sites [41]. Meanwhile, enhanced PECAM1 signaling could also promote macrophage migration, as it was reported to be essential for leukocyte transendothelial migration under most inflammatory conditions [42]. However, PECAM1 could act as a negative feedback regulator of inflammation, as evidenced by its ability to inhibit LPS-induced inflammation through TLR4 [43].

The complicated interactions among immune cells, involving a myriad of pathways, presented a holistic tableau in which B cells

ANNEXIN signaling pathway



PECAM1 signaling pathway

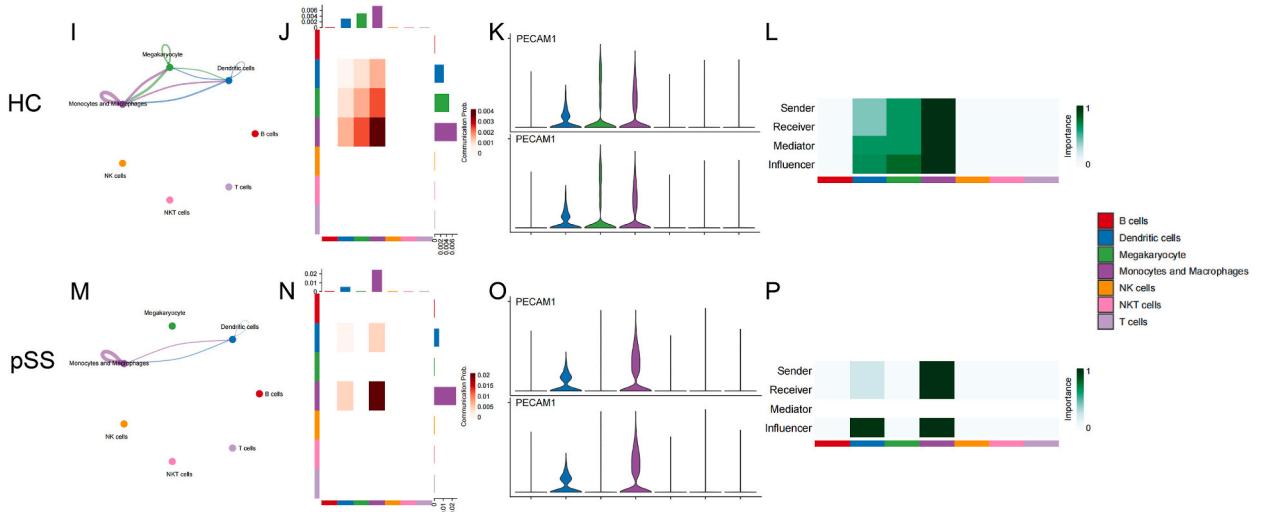


Fig. 10. The intercellular signal strength, the intensity of the signals sent and received by each cell type, the expression of receptors and ligands on the cell surface, and the role of each cell type in the signaling network of the signaling pathways with high incoming and outgoing signals in monocytes/macrophages between HC and pSS patients (A)–(H) ANNEXIN signaling pathway (I)–(P) PECAM1 signaling pathway.

and T cells might have been stimulated by monocytes/macrophages, and the adhesion and aggregation might have been enhanced between them. The alterations in the signaling network of monocytes/macrophages suggested that they were not only direct effectors but also adept communicators among immune cells, potentially fueling the enduring state of chronic inflammation observed in pSS.

The main limitation of this study stems from the absence of experimental evidence, partly attributed to the study’s overarching focus on the overall immune cell landscape, while subsequent experiments that delve deeper into specific molecular components would be beneficial. Another limitation is the number of participants qualified for the study, which is due to the impracticality of large-scale application of scRNA sequencing. In addition, problems such as horizontal pleiotropy and weak IVs in MR analysis may lead to unrobust results. Although we used some methods to eliminate unqualified IVs, such as MR-PRESSO test and F-value, to reduce the risk when performing MR Analysis, there is still a risk of potential bias. Batch effects are another issue that deserves attention. Despite efforts to reduce the impact of the batch effect, latent risks may also exist.

In summary, the study underscored the pivotal contribution of monocytes/macrophages to pSS pathogenesis, substantiated by both Mendelian randomization analysis and comprehensive cell-to-cell interaction assessment. However, the absence of direct evidence definitively establishing macrophage function in pSS pathogenesis remains a non-negligible gap. In light of this, monocytes/macrophages are anticipated to be a focal point for pathogenesis research and potential therapeutic targeting in pSS.

Table 3
Changes of monocytes/macrophages-related intercellular signaling pathways.

Pathways	Intercellular interaction	Signal strength	Roles in signal networks	Figure
High Outgoing Signaling				
ADGRE5	New: Mono/Mac signaling to NK cells and receiving signals from T cells	Heightened: Outgoing signaling toward B cells; Self-signaling	Intensified: Senders Influencers	Figs. 8–1 A–H
ICAM	New: Mono/Mac signaling to T cells and receiving signals from B cells	Heightened: Incoming signaling from B cells and T cells	Intensified: Senders Receivers Influencers	Figs. 8–1 I–P
RESISTIN	New: Mono/Mac signaling to DCs, B cells, T cells and NK cells; Mono/Mac self-signaling Absent: Mono/Mac receiving signals from DCs	Heightened: Outgoing signaling toward DCs, B cells, T cells and NK cells; Self-signaling	Intensified: Senders Receivers Influencers	Figs. 8–2 A–H
BAFF	/	Heightened: Outgoing signaling toward B cells	Intensified: Senders	Figs. 8–2 I–P
High Incoming Signaling				
GALECTIN	/	Heightened: Outgoing signaling toward DCs, B cells, T cells, NK cells and NKT cells; Self-signaling	Intensified: Senders Receivers	Fig. 9A–H
SEMA4	Absent: Mono/Mac receiving signals from T cells, NK cells and NKT cells	Attenuated: Incoming signaling from T cells, NK cells and NKT cells	Weakened: Senders	Fig. 9I–P
High incoming and outgoing signals				
ANNEXIN	/	Heightened: Incoming signaling from DCs, T cells and NK cells; Self-signaling	Intensified: Senders Receivers Mediators Influencers	Fig. 10A–H
PECAM1	/	Heightened: Self-signaling	Weakened: Mediators	Fig. 10I–P

CRedit authorship contribution statement

Yimei Ding: Writing – original draft, Methodology, Formal analysis, Data curation. **Xue Luan:** Writing – review & editing, Supervision. **Jiaqi Hou:** Writing – original draft, Validation, Funding acquisition.

Data availability statement

The scRNA-seq data used in this study can be obtained from GEO database under the accession number GSE157278. The GWAS data used in this study can be obtained from the openGWAS database with the GWAS ID finn-b-M13_SJOGREN.

Ethics approval

Review and/or approval by an ethics committee was not needed for this study because all data in this study were derived from public databases. Appropriate ethics approval has been provided at the publication of original articles. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jiaqi Hou reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

pSS	primary Sjögren's syndrome
MR	Mendelian randomization
DEG	differentially expressed gene
eQTL:	expression quantitative trait loci
pQTL:	protein quantitative trait loci
NK	natural killer
scRNA-seq	single-cell RNA sequencing
PBMC	peripheral blood mononuclear cell
HC	healthy controls
DC	dendritic cell
GWAS	genome-wide association
IV	instrumental variable
IVW	inverse-variance weighted
SNP	nucleotide polymorphism
OR	odds ratio
APC	antigen-presenting cell
MHC-II	histocompatibility complex class II

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e39130>.

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