Observational Study

Medicine

OPEN

Bioinformatics analysis identified immune infiltration, risk and drug prediction models of copper-induced death genes involved in salivary glands damage of primary Sjögren's syndrome

Naidan Zhang, MS[a], Chaixia Ji, MS[a], Xinyin Peng, MS[b], Maoju Tang, MS[c], Xiao Bao, MS[d] and Chengliang Yuan, PhD[a,*]

Abstract

This study aimed to identify copper-induced death genes in primary Sjögren's syndrome (pSS) and explore immune infiltration, risk and drug prediction models for salivary glands (SGs) damage. The 3 datasets, including GSE40611, GSE23117, and GSE7451 from the Gene Expression Omnibus database were downloaded. The datasets were processed using the affy in R (version 4.0.3). In immune cells, copper-induced death genes were strongly expressed in "activated" dendritic cells (aDCs), macrophages and regulatory T cells (Treg). In immune functions, copper-induced death genes were strongly expressed in major histocompatibility complex (MHC) class I, human leukocyte antigen (HLA) and type I interferon (IFN) response. Correlation analysis showed that 5 genes including *SLC31A1*, *PDHA1*, *DLD*, *ATP7B*, and *ATP7A* were significantly correlated with immune infiltration. The nomogram suggested that the low expression of *PDHA1* was significant for predicting the risk of pSS and the area under curve was 0.678. Drug model suggested that "Bathocuproine disulfonate CTD 00001350," "Vitinoin CTD 00007069," and "Resveratrol CTD 00002483" were the drugs most strongly associated with copper-induced death genes. In summary, copper-induced death genes are associated with SGs injury in pSS, which is worthy of clinicians' attention.

Abbreviations: aDCs = "activated" dendritic cells, ATP7A = ATPase copper transporting α, ATP7B = ATPase copper transporting β, ATRA = all-trans retinoic acid, CCR = CC chemokine receptors, DLD = dihydrolipoamide dehydrogenase, HLA = human leukocyte antigen, IFN = type I interferon, MHC = major histocompatibility complex, PDHA1 = pyruvate dehydrogenase E1 subunit α1, pSS = primary Sjögren's syndrome, Res = Resveratrol, ROC = receiver operating curve, ROR-γ = receptor-related orphan nuclear receptor γ, SGs = salivary glands, SLC31A1 = solute carrier family 31 member 1, TIL = tumor infiltrating lymphocytes, Treg = regulatory T cells.

Keywords: copper-induced death genes, immune infiltration, prediction models, primary Sjogren's syndrome, salivary glands damage

1. Introduction

Xerostomia is one of the common clinical manifestations of Sjögren's syndrome. It is mainly caused by impaired salivary glands (SGs) secretion, resulting in rampant caries, parotid gland enlargement and angular cheilitis.[1–3] Copper, as one of the essential trace elements, plays an important role in iron transport, expression of vascular endothelial growth factor and angiogenesis.[4–6] Excessive doses of copper can cause overexpression of genes associated with cell death and damage immune system through the EndoG-Bax-ubiquitin pathway.[7] At present, the pathogenesis of copper-induced death genes in SGs of primary Sjögren's syndrome (pSS) is not clear. Immune factors and genetic background are considered to be the basis of the occurrence of pSS.[8,9]

By studying genes and immune cells associated with SGs in pSS, central genes such as CD38, CMPK2, TBC1D9, and PYCR1 were associated with the immune infiltration in SGs, mitochondrial metabolic pathway in gluconeogenesis and tricarboxylic acid cycle.[10] Copper oxide quantum dots significantly reduced the viability of C2C12 cells in a concentration-dependent manner (10–20 μg/mL) and inhibited mitochondrial caspase 3 and 7 by binding to DNA.[11] Further studies showed that copper-dependent apoptosis depended

This work was supported by Sichuan Medical Association (grant number: 2019HR35) and Southwest Medical University (grant number: ZYTS-130).

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Clinical Laboratory, Peoples Hospital of Deyang City, Deyang, China, [b] Chengdu University of Chinese Medicine, Chengdu, China, [c] North Sichuan Medical College, Nanchong, China, [d] Department of Rheumatology, Peoples Hospital of Deyang City, Deyang, China.

**Correspondence: Chengliang Yuan, Department of Clinical Laboratory, Peoples Hospital of Deyang City, North Taishan Road No. 173, Deyang, Sichuan 618000, China (e-mail: 283072302@qq.com).*

How to cite this article: Zhang N, Ji C, Peng X, Tang M, Bao X, Yuan C. Bioinformatics analysis identified immune infiltration, risk and drug prediction models of copper-induced death genes involved in salivary glands damage of primary Sjögren's syndrome. Medicine 2022;101:41(e31050).

Received: 19 May 2022 / Received in final form: 6 September 2022 / Accepted: 8 September 2022

http://dx.doi.org/10.1097/MD.0000000000031050

not only on concentration, but also on type of cell and time of exposure.[12] Studies on apoptosis and pSS suggested that anti-cholinergic autoantibodies mediated apoptosis of the A253 cell line in a dependent manner of inositol phosphate, caspase-3 and matrix metalloproteinase-3.[13] These findings suggested that the expression of copper-induced death genes were related to the disorder of immune microenvironment in SGs in pSS.

As far as we know, studies on copper-induced death genes with immune infiltration in pSS are relatively rare, especially using copper-induced death genes to build risk prediction model and drug model. Therefore, we studied from the perspective of copper-induced death genes and immune infiltration as well as prediction and drug models. This is a new experiment of copper-induced death genes in pSS, and may help clinicians to identify potential biomarkers for predicting and diagnosing pSS.

2. Materials and methods

2.1. Selection and expression matrix extraction of copper-induced death genes

Study flowchart was showed in Figure 1. Three pSS datasets (GSE7451, GSE23117, GSE40611) were downloaded from the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). All the datasets were pre-processed by affy in R (version 4.0.3), including data merge, normalization, and log2 transformation. In order to explore the interaction between genes, we used weighted gene co-expression network analysis to construct gene co-expression network. To ensure the reliability of network construction results, we removed outlier samples and introduced genes with more than 25% variation in the integrated dataset into weighted gene co-expression network analysis.

In previous studies, genome-wide CRISPR-Cas9 deficiency screening was used to identify 13 genes involved in copper-induced death, including ATPase copper transporting α (*ATP7A*), ATPase copper transporting β (*ATP7B*), copper importer SLC31A1 (*CTR1*), dihydrolipoamide dehydrogenase (*DLD*), dihydrolipoamide S-acetyltransferase, dihydrolipoamide S-succinyltransferase, dihydrolipoamide branched chain transacylase E2, ferredoxin 1, glycine cleavage system protein H, lipolytransferase 1, lipoyl synthase, pyruvate dehydrogenase E1 subunit α1 (*PDHA1*), pyruvate dehydrogenase E1 subunit β and solute carrier family 31 member 1 (*SLC31A1*).[14]

2.2. Correlation analysis on copper-induced death genes and immune infiltration

To evaluate the role of copper-induced death genes in the immune microenvironment, we analyzed the correlation of copper-induced death genes and immune microenvironment in SGs of pSS patients by gene expression matrix. The GSVA package was used to calculate the enrichment of immune infiltration including 16 immune cells and 13 immune functions. The pheatmap package was used to show the concentration of copper-induced death genes in immune cells and immune functions.

The corrplot package was used to analyze the correlation of 16 infiltrating immune cells and 13 immune functions, respectively. We used correlation coefficient to evaluate the correlation between immune cells and immune function, respectively. According to the correlation coefficient, we classified the correlation of immune cells and immune functions from strong to weak. We used a plus sign to indicate a positive correlation and a minus sign to indicate a negative correlation.

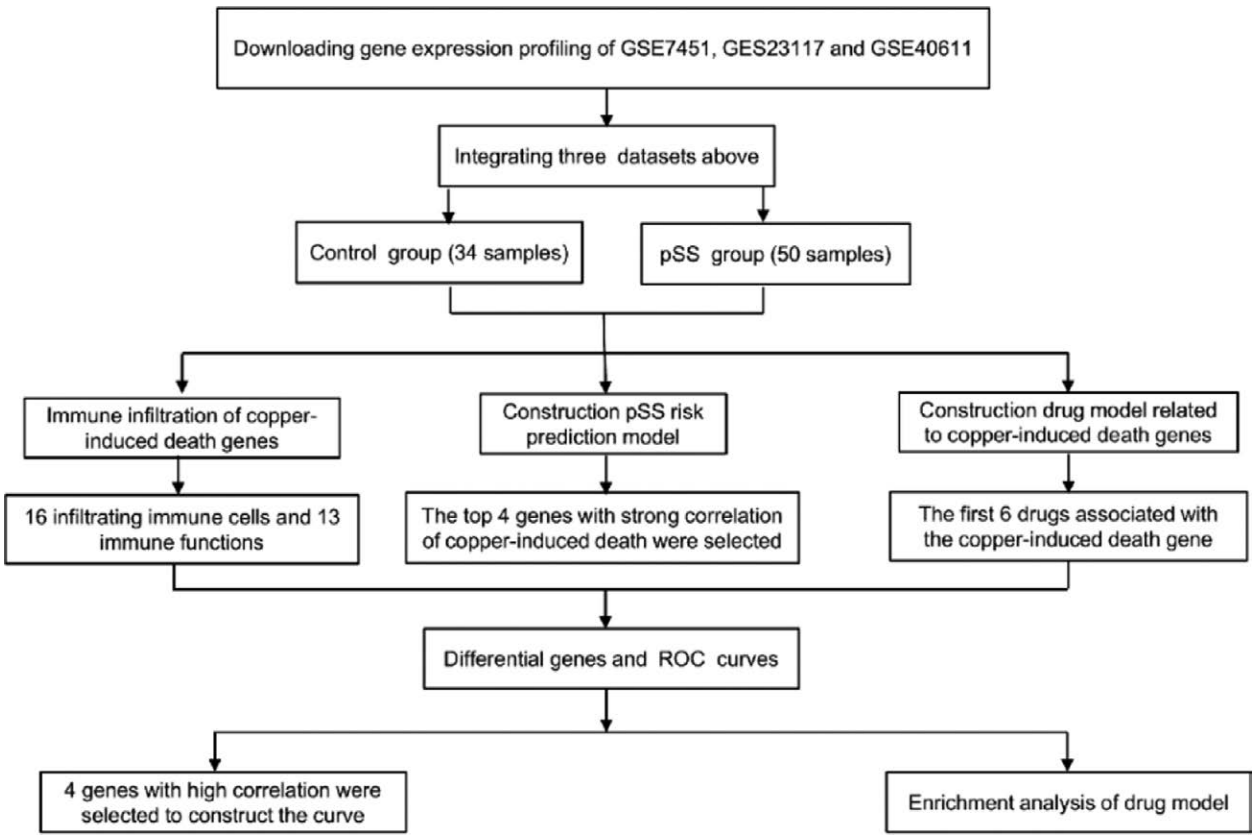

Figure 1. Study flowchart. Abbreviations: pSS = primary Sjogren's syndrome; ROC = receiver operating curve.

2.3. Difference in immune score between health controls and pSS

The reshape2 package was used to transform data to calculate the difference of immune score between healthy controls and pSS. The ggpubr package was used to plot the differences between the 2 groups, a P value <.05 was considered statistically significant.

To screen out copper-induced death genes associated with immune infiltration, we used psych package to correlate the immune infiltration expression matrix with the copper-induced death gene expression matrix. The ggcorrplot package was used to calculate correlation coefficients and P values. A P value <.05 was considered statistically significant. If the |correlation coefficient| <.5, the correlation was weak, and if the |correlation coefficient| was ≥.50, the correlation was medium or strong.

2.4. Construction pSS risk prediction model based on copper-induced death genes

Through the screening of copper-induced death genes related to immune infiltration, we selected the top 4 genes with strong correlation to construct risk prediction model of pSS. The rms package was used to draw nomogram and show the relationship between each variable in the prediction model. Calibration curves were plotted to assess the calibration of the nomogram. The receiver operating curve (ROC) was constructed to predict the risk of pSS. Area under curve was obtained by ROC curve. A P value <.05 was considered statistically significant.

2.5. Enrichment analysis of drug model related to copper-induced death genes

The drug model of copper-induced death genes was constructed with gene enrichment analysis tool (https://maayanlab.cloud/Enrichr/) and the adjust P value <.05 was defined as the cut-off value.[15] We downloaded the therapeutic drug information related to copper-induced death genes from the above website. The clusterProfiler and ggplot2 packages were used for gene and drug enrichment analysis of copper-induced death genes screened in this study. The significant enrichment for analyses threshold was adjust P value <.05. According to the adjust P values, the first 6 drug names were selected and displayed.

3. Results

3.1. Expression of copper death-related genes in immune infiltration

A total of 16 immune cells and 13 immune functions were differentially expressed. Infiltration scores of pSS were obtained by the ssGSEA method. In immune cells, copper-induced death genes were strongly expressed in "activated" dendritic cells (aDCs), macrophages and regulatory T cells (Treg). In immune functions, copper-induced death genes were strongly expressed in major histocompatibility complex (MHC) class I, human leukocyte antigen (HLA) and type I interferon (IFN) response. Among them, copper-induced death genes had the highest expression intensity in immune functions of MHC class I, and were associated with abnormal expression of β-2-Microglobulin, HLA-A and transporter 1. The heatmap of immune cells and immune functions were showed in Figure 2.

3.2. Correlation analysis of immune infiltration

Correlation analysis showed the top 3 correlation in immune cells were tumor infiltrating lymphocytes (TIL) and B cells, TIL and follicular helper T, B cells and follicular helper T. The top 3 correlation in immune functions were T cell co-stimulation and check-point, CC chemokine receptors (CCR) and check-point, T cell co-inhibition and check-point, as showed in Figure 3A and B.

The boxplots of differences between immune score in health controls and pSS were illustrated in Figure 4. In immune cells, aDCs (.657 vs 594, P < .001), TIL (.549 vs 492, P < .001), Treg (.621 vs 598, P < .001), neutrophils (.550 vs 489, P = .001), pDCs (.478 vs 445, P = .001), B cells (.492 vs 434, P = .004), Th2 cells (.500 vs 470, P = .004), T helper cells (.399 vs 327, P = .006) and macrophages (.631 vs 601, P = .010) were significantly increased in pSS. In immune functions, HLA (.793 vs 743, P < .001), MHC class I (.916 vs 873, P < .001), inflammation-promoting (.545 vs 478, P < .001), parainflammation (.645 vs 601, P < .001) and type I IFN reponse (.728 vs 625, P < .001) were significantly higher in pSS than controls. Antigen presenting cell (APC) co inhibition (.657 vs 466, P = .009), T cell co-inhibition (.466 vs 439, P = .032) and cytolytic activity (.614 vs 559, P = .014) were also higher in pSS, as shown in Figure 3C and D.

3.3. Correlation between copper-induced death genes and immune infiltration

Correlation analysis of copper-induced death genes and immune infiltration in pSS patients showed that among the 12 copper-induced death genes, 5 genes were significantly correlated with immune infiltration, including *SLC31A1*, *PDHA1*, *DLD*, *ATP7B*, and *ATP7A*. The *DLD* and *ATP7A* were positively correlated with immune infiltration while *SLC31A1*, *PDHA1*, and *ATP7B* were negatively correlated with immune infiltration (P < .05). *SLC31A1* and *PDHA1* were associated with the abnormality of various immune cells and immune functions in pSS, especially Th1 cells, CCR and T cell co – inhibition, as shown in Figure 4A.

3.4. Copper-induced death genes construct ROC curves

According to the correlation between copper-induced death genes and immune infiltration, the top 4 genes were selected to construct ROC curve and the risk of pSS were predicted. The copper-induced death genes were divided into high and low expression by the median expression value. According to the nomogram, the results suggested that the expression of *PDHA1* was significant for predicting the risk of pSS, as shown in Table 1. When expression of *PDHA1* was low, the total score was near 180 and the risk of pSS was greater than 80%, as shown in Figure 4B. ROC curve indicated that the area under curve was 0.654, indicating that this model had a good predictive ability for pSS, as shown in Figure 4C.

3.5. Enrichment analysis of copper-induced death gene related drug model

A total of 175 drug-related messages of copper-induced death gene (*SLC31A1*, *PDHA1*, *DLD*, and *ATP7B*) were downloaded from gene enrichment analysis tool. After screening through adjust P value <.05, a total of 71 drug information were included. Gene enrichment analysis showed that the drugs were mainly involved in *SLC31A1* term, including "CHEMBL1182312 CTD 00004324," "cytochalasin D CTD 00007076," and "Trimethyl – beta – cyclodextrin CTD 00003512." In *PDHA1* term, the drugs were significantly enriched in "Vitinoin CTD 00007069," "2, 6 – DICHLORO – 4 – NITROPHENOL CTD 00000815," and "resveratrol CTD 00002483." DLD term showed that the drugs were mainly enriched in "Isoquercitrin TTD 00008703," "RUTIN TTD

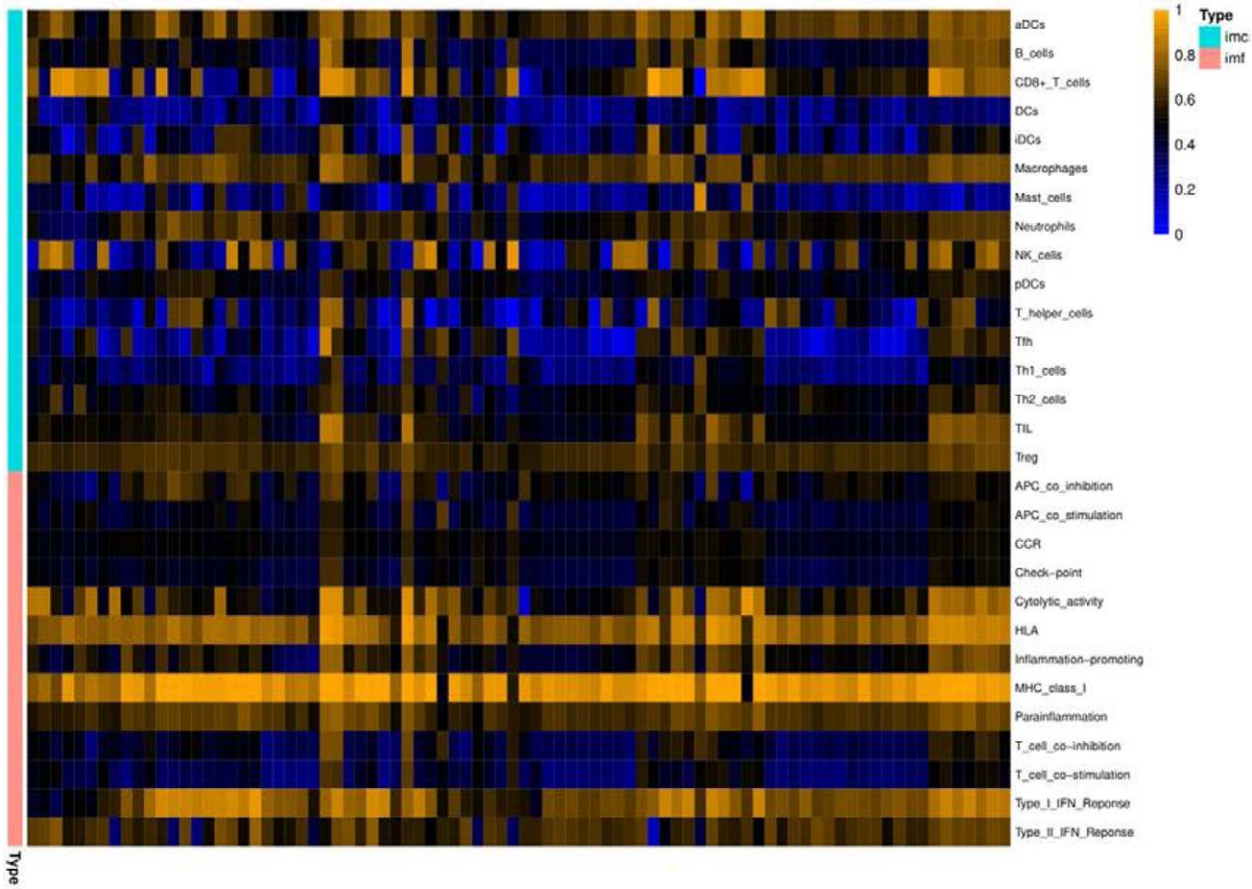

Figure 2. Heatmap of copper death-related genes in immune cells and immune functions. Light blue represents 16 types of immune cell, orange represents 13 types of immune function. Orange represents copper death-related genes are significantly related to immune infiltration, blue represents those are not related to immune infiltration. Abbreviations: aDCs = "activated" dendritic cells, APC = antigen presenting cell, CCR = CC chemokine receptors, IFN = interferon, imc = immune cell, imf = immune function, MHC = major histocompatibility complex, NK = natural killer, Tfh = follicular helper T, Th = helper T cell, TIL = tumor infiltrating lymphocytes, Treg = regulatory T cells, HLA = human leukocyte antigen.

00010730," "Indatraline hydrochloride TTD 00008587," "5, 7 – Dimethoxyflavone CTD 00002502," "brimonidine CTD 00000810," and "flavin adenine dinucleotide TTD 00008045." Additionally, ATP7B term was found to be enriched in "Bathocuproine disulfonate CTD 00001350," "MG – 132 CTD 00002789," and "CID755673 CTD 00004896," as shown in Figure 5A.

We downloaded the figure of copper-induced death gene related drug and verified the drug use frequency chart, as shown in Figure 5B. The results suggested that "Bathocuproine disulfonate CTD 00001350", "Vitinoin CTD 00007069" and "Resveratrol CTD 00002483" were the drugs most strongly associated with copper-induced death genes. It was consistent with the results of this study.

4. Discussion

pSS is a chronic inflammatory autoimmune disease characterized by specific pathological changes. Previous studies on pSS and genes mainly focused on screening differentially expressed genes, correlation analysis between signaling pathways, genes and protein interaction network.[16–18] There are few studies on copper-induced death genes, immune infiltration score and prediction model construction.[19] In this study, we found that copper-induced death genes were associated with a variety of immune cells and immune functions. Copper-induced death genes were significantly expressed in aDCs, macrophages, and Treg in immune cells. They were strongly expressed in immune functions such as MHC class I, HLA and type I IFN reponse. In early studies, CD4 + cytotoxic T lymphocytes (CTL) and DCs may be involved in the proliferation of activated B lymphocytes in SGs of pSS.[20] Further studies showed that RNA interacted with Fcγ receptor IIa and triggered the activation of RNA-containing immune complexes in plasmacytoid dendritic cells (pDCs).[21]

Expression of C-C chemokine receptor 5 (CCR5) and its ligands C-C chemokine ligand type 3 and CCL4 in SGs played an important role in effective migration of DCs.[22,23] DCs recognized NK cells that binded to B7-H6 in SG epithelial cells and secreted Th1 cytokines such as IFN-γ and interleukin (IL)-12.[24,25] In this study, it was found that aDCs and pDCs of pSS were significantly increased, while DCs and iDCs were not significantly different. This was an important complement to DCs in the pathogenesis of pSS. In this study, copper-induced death genes were also highly expressed in MHC I, HLA and type I IFN reactions. Copper-induced death genes may also be related to abnormal activation of DCs in SGs, and the specific subtypes of activated dendritic cells need to be verified in further experiments.

Macrophage was the main leukocytes in tissues and the phenotypic characteristics were closely related to the immune microenvironment. Macrophages were activated by IFN-γ and IL-17 from Th1 and Th17 cells in SGs of pSS.[26] Activated macrophages were mainly involved in the pathogenesis of pSS in the following 2 ways: one is to produce inflammatory cytokines such as IL-1, tumor necrosis factor α, IL-18 and metalloproteinases, which leaded to epithelial cell damage.[27] Another is

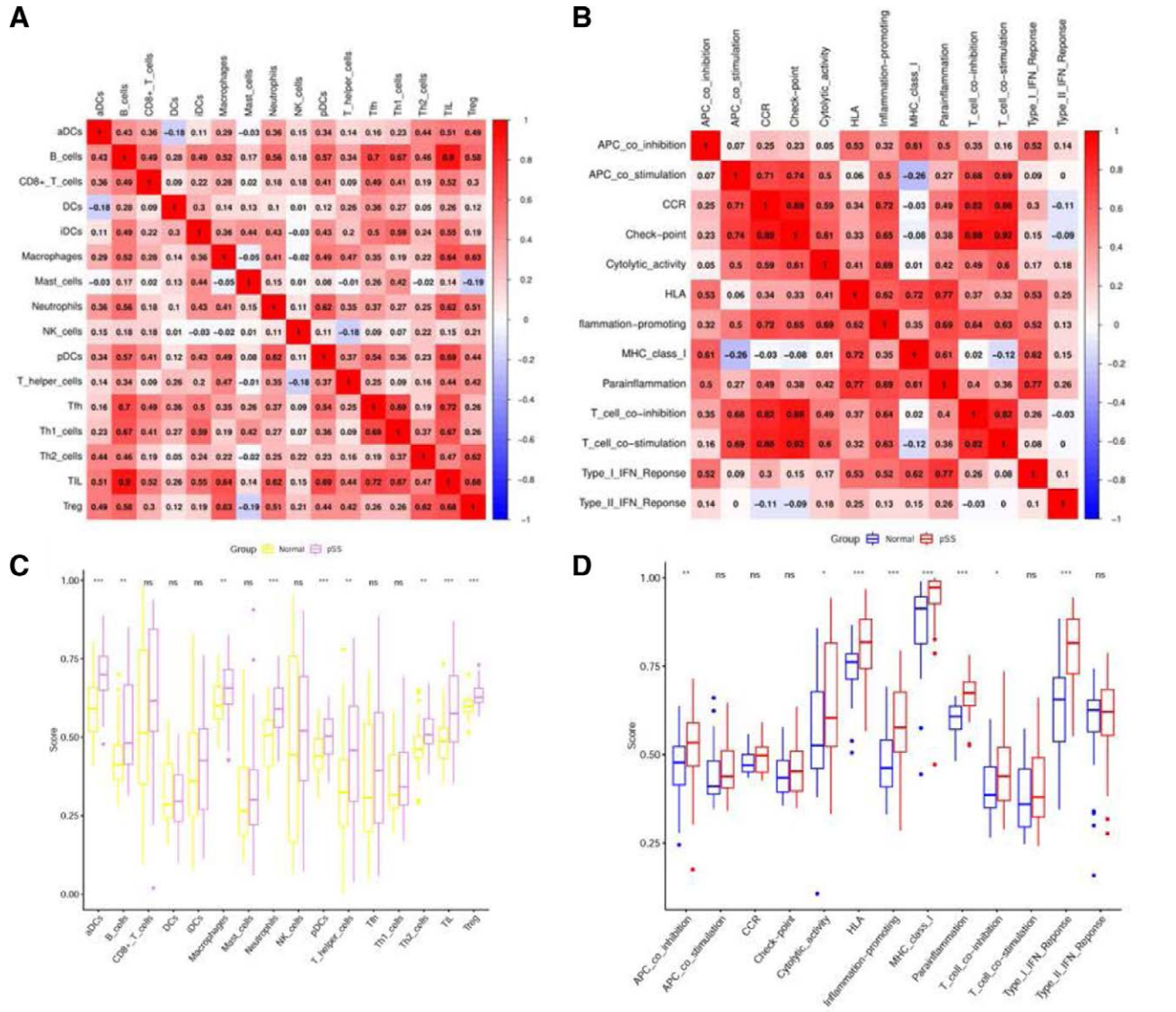

Figure 3. Correlation analysis of immune infiltration and comparison between immune score in pSS and controls. (A) Correlation analysis of 16 types of immune cells. (B) Correlation analysis of 13 types of immune functions, red and blue represent strong positive and negative correlation respectively, while white represents no correlation. (C) Comparison of 16 types of immune cells, horizontal axes demonstrates immune cell subtypes, vertical axes demonstrates immune score (yellow represent control and violet represent pSS). (D) Comparison of 13 types of immune functions, horizontal axes demonstrates immune functions, vertical axes demonstrates immune score (blue represent control and red represent pSS). *P < .05; **P < .01; ***P < .001. Abbreviations: aDCs = "activated" dendritic cells, APC = antigen presenting cell, CCR = CC chemokine receptors, HLA = human leukocyte antigen, IFN = interferon, MHC = major histocompatibility complex, Tfh = follicular helper T, Th = helper T cell, TIL = tumor infiltrating lymphocytes, Treg = regulatory T cells.

to activate CD4 + T cells through MHC-II as antigenic peptide presenting cells. The 2 interacted to maintain the pro-inflammatory automatic maintenance cycle.[28]

Early studies showed that Treg could effectively inhibit the activation of self-reactive T cells, further avoiding the accelerated maturation of DCs.[29] Another study showed that labial gland-derived mesenchymal stem cells effectively inhibited the differentiation of Th17 cells and induce the differentiation into Treg cells. Labial gland-derived mesenchymal stem cells educed the secretion of IL-17, IFN-γ and IL-6, and restored SG secretion function.[30] Our study showed that macrophages, TIL, Th2 and Treg in pSS were significantly increased. TIL and Treg were associated with abnormal activation of various immune cells. These results suggested that DCs and macrophages were closely associated with abnormal activation of T cells in SGs of pSS. Although the role of Treg in pSS is still controversial.[31,32] It is worth further studies in the activation of macrophages and DCs and relationship with pSS.

In this study, immune functions of pSS were characterized by MHC class I, HLA, and type I IFN responses. This result was also reported in the previous studies. Levels of HLA-DR were elevated in exocrine epithelial cells of pSS, inducing activation and infiltration of CD4 + T cells.[33] Recent studies using microarrays to analyze the expression of SGs also found that gene expression patterns in pSS involve multiple chronic inflammatory pathways, such as chemokines, cytokines, MHC, and IFN.[34–36] This study showed that copper-induced death genes were mainly related to the inflammatory response of pSS. We speculated that copper-induced death genes were associated with immune infiltration in SGs of pSS patients. It may provide important clues for the treatment of pSS.

We also explored the relationship between copper-induced death genes and the risk of pSS, which was an innovative attempt. In this study, we found that *SLC31A1* and *PDHA1* were associated with various immune cells and immune functions in pSS. It was suggested that excessive copper could target

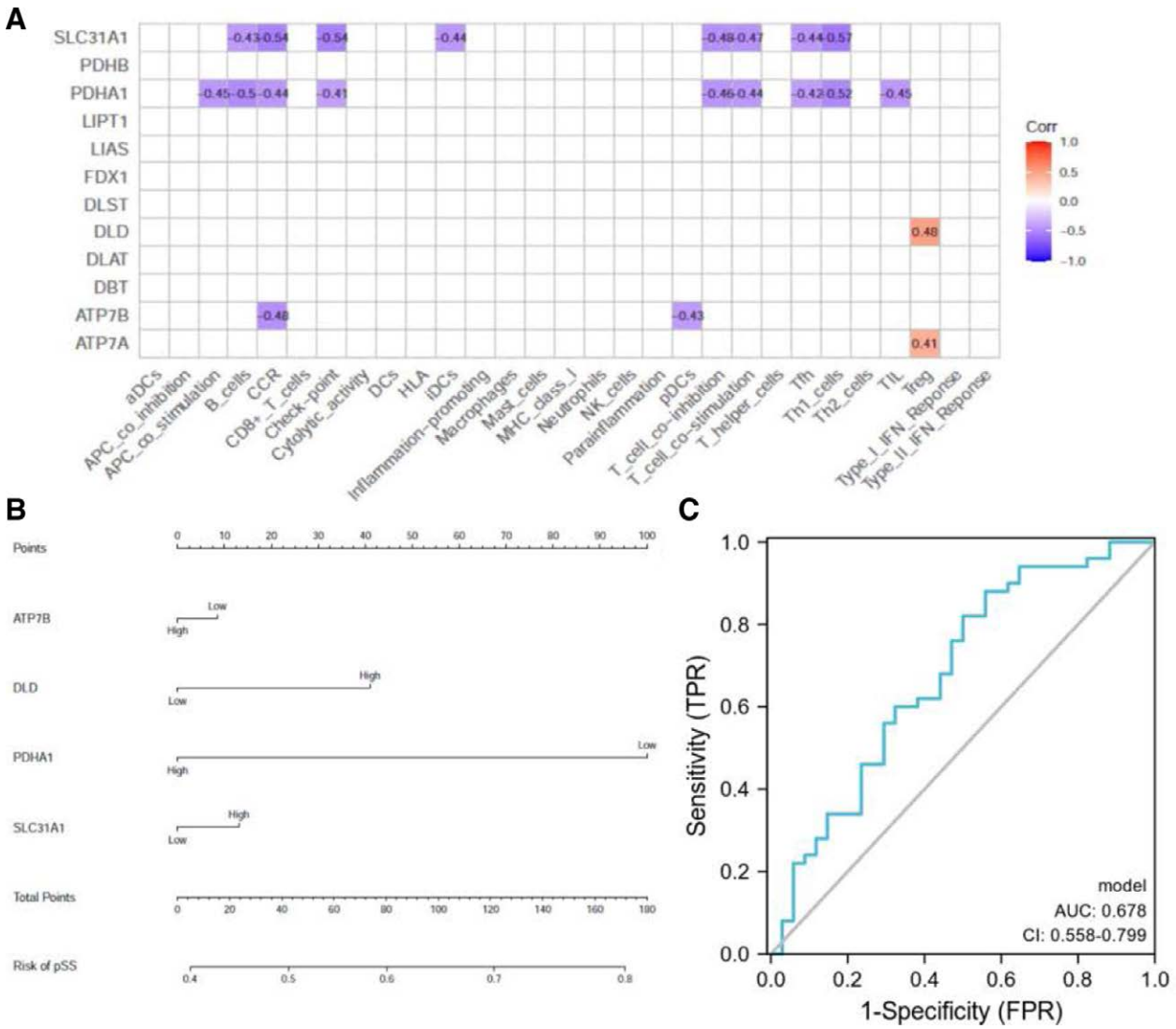

Figure 4. Diagnostic efficacy of copper death-related genes. (A) Correlation of copper death-related genes and immune infiltration, horizontal axes demonstrates immune infiltration, vertical axes demonstrates copper death-related genes. (B) Column map of top 4 genes, horizontal axes demonstrates risk of pSS, vertical axes demonstrates genes. (C) Diagnostic efficacy of combined prediction model. Abbreviations: AUC = area under curve, CI = confidence Interval, FPR = false positives ratio, pSS = primary Sjogren's syndrome, TPR = true positives ratio.

Table 1

ROC curve of differentially expressed genes.

Predictor variable	AUC	Sensitivity	Specificity	95%CI	Standard Error	*P*
ATP7B	.538	.740	.500	.402-.674	.486	.851
DLD	.598	.420	.794	.473-.722	.477	.356
PDHA1	.655	.960	.441	.519-.791	.519	.038*
SLC31A1	.464	.588	.632	.336-.591	.516	.786
Combined 4 genes	.678	.880	.441	.558-.799	.453	.118

AUC = area under the curve, ROC = receiver operating characteristic.
*$P < .05$.

to induce tumor cell death in tumor therapy.[14] It was suggested that the role of copper-induced death genes in pSS may be inconsistent. Low expression of *PDHA1* gene was closely associated with risk of pSS, while high expression of *DLD* gene was associated with risk of pSS. *PDHA1* gene encoded the E1 subunit α 1 of pyruvate dehydrogenase and played a key role in pyruvate dehydrogenase complex.[37] Inhibition of *PDHA1* expression in human esophageal squamous cell cancer significantly reduced oxidative phosphorylation, leading to increased angiogenesis and malignancy.[38] In this study, we found that the low expression of *PDHA1* in pSS had biological significance and was associated with the risk of pSS. It is an extension of the research field of *PDHA1* gene. More studies on the metabolic reprogramming of *PDHA1* and its protein expression products may provide potential therapeutic targets for pSS.

We also attempted to conduct enrichment analysis of copper-induced death genes in therapeutic models, and verified by relevant websites. Results showed that "bathocuproine disulfonate," "vitinoin," and "resveratrol" were the drugs most strongly associated with copper-induced death genes. Retinoic acid was the active metabolite of vitamin A, and its main active ingredient was all-trans retinoic acid (ATRA). ATRA produced by DCs promoted differentiation of T cells into FoxP3 + T cells and inhibit differentiation of Th17. The combination of ATRA and TGF-β transferred the balance of Treg/Th17 and reduced the immune inflammatory response.[39] ATRA regulated the expression of FoxP3 gene and induced the proliferation and differentiation of Treg in CD4 + T cells.[40] Retinoic acid receptor α and -γ were

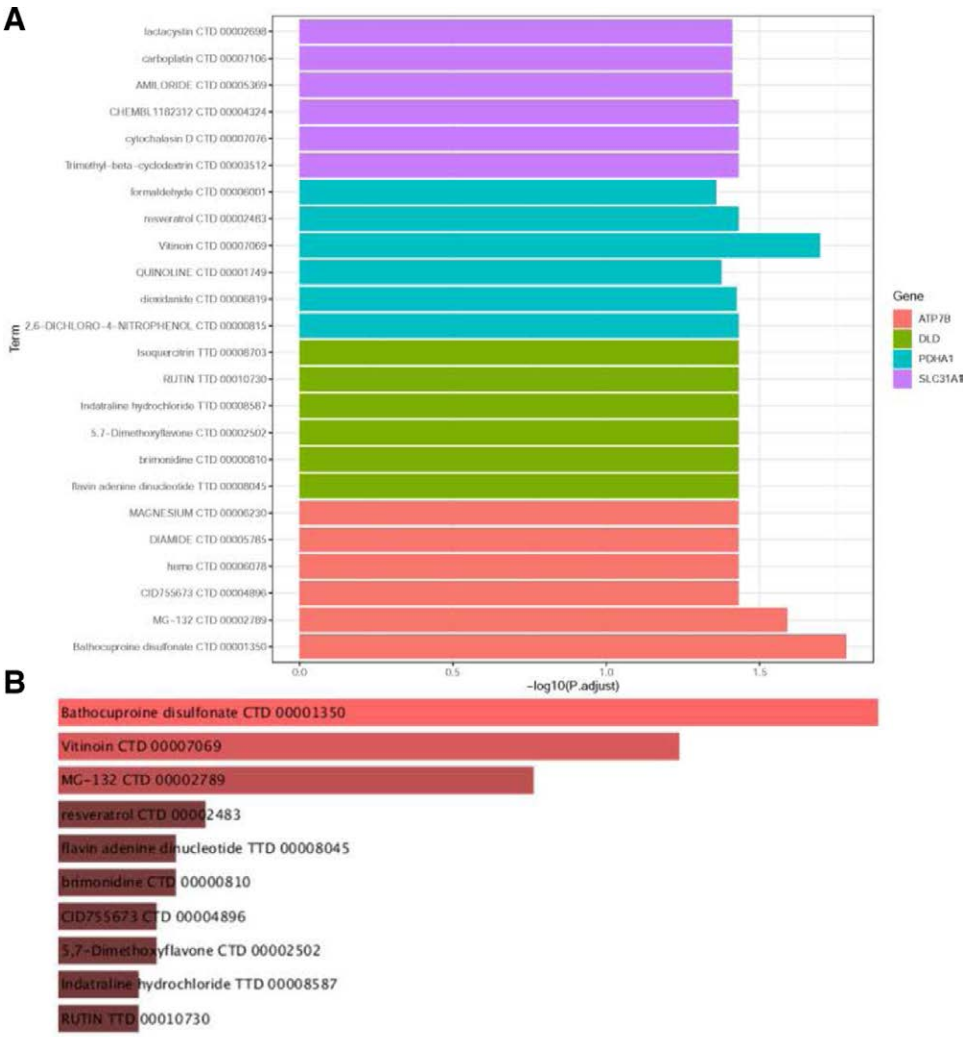

Figure 5. Enrichment analysis of drug model related to copper-induced death genes. (A) Construct drug model of copper-induced death gene, horizontal axes demonstrates 4 genes, vertical axes demonstrates top 6 drugs with the highest frequency of copper-induced death genes. (B) Drug use frequency chart of copper-induced death genes.

expressed on the surface of T cells. ATRA also promoted FoxP3 $^{+}$ T cell expression by activating CD4 $^{+}$ T cells with Retinoic acid receptor α.[41,42] ATRA inhibited Th17 mainly by binding receptor-related orphan nuclear receptor γ (ROR-γ) to exert anti-inflammatory effect.[43] In mouse model, overexpression of ROR-α not only attenuated ROR-γ and IL-17, but also regulated production of tumor necrosis factor-α, improving the inflammatory environment.[44] IL-6 can induce high levels of ROR-γ and up-regulate IL-23R, finally promoted the production of IL-17 through the signal transducers and activators of transcription 3 pathway.[45,46] ATRA down-regulated IL-6Rα and IL-23R and played an important role in differentiation of Th17.[47,48]

Resveratrol (Res) is a natural non-flavonoid polyphenol compound, which has strong immunomodulatory and anti-inflammatory effects.[49] Res increased IL-10 and deacetylase 1, and effectively improved the salivation dysfunction in non-obese diabetic mice.[50] In addition, Res reduced dextran sodium sulfate-induced IBD by regulating small ubiquitin-like modifier protein 1 through the Wnt/β-catenin pathway. The expression of anti-inflammatory cytokines increased and pro-inflammatory cytokines decreased in colon and spleen tissues of mice.[51] Since there are few studies on Res and pSS, we speculate that whether drugs based on copper-induced death gene needs to be verified by further models.

Our study has some limitations. First, there are only a few genes related to copper-induced death that can be found at present, and the potential genes related to pSS may be neglected. Second, further studies on immune infiltration are needed to screen potential risks and drug treatment models of pSS.

5. Conclusion

We hypothesized that copper-induced death genes may lead to SGs damage in pSS by influencing immune infiltration disorder. *SLC31A1*, *PDHA1*, *DLD*, *ATP7B*, and *ATP7A* were significantly associated with abnormal expression of immune infiltration and may be important genes in the regulation of pSS. *PDHA1* gene has a high predictive value for the risk of pSS. In addition, ATRA and Res may have certain reference value for drug treatment of pSS.

Acknowledgments

We wanted to express our gratitude to the providers of the datasets GSE7451, GSE23117 and GSE40611 for sharing the datasets.

Authors' contributions

Conceptualization: Chengliang Yuan.
Formal analysis: Naidan Zhang.
Funding acquisition: Naidan Zhang, Chengliang Yuan.
Investigation: Naidan Zhang, Maoju Tang.
Methodology: Chaixia Ji, Chengliang Yuan.
Project administration: Naidan Zhang, Chengliang Yuan.
Resources: Xiao Bao.
Software: Naidan Zhang.
Supervision: Xinyin Peng.
Writing – original draft: Naidan Zhang.
Writing – review & editing: Chengliang Yuan.

References

[1] Mori G, Kobayashi T, Ito T, et al. Implant-supported prostheses in patient with Sjögren's Syndrome: clinical Report with 3-year Follow-up. Bull Tokyo Dent Coll. 2018;59:201–6.
[2] Zhang X, Feng R, Zhao J, et al. Salivary gland ultrasonography in primary Sjögren's syndrome from diagnosis to clinical stratification: a multicentre study. Arthritis Res Ther. 2021;23:305.
[3] Serrano J, Lopez-Pintor RM, Gonzalez-Serrano J, et al. Oral lesions in Sjögren's syndrome: a systematic review. Med Oral Patol Oral Cir Bucal. 2018;23:e391–400.
[4] Tavsan Z, Ayar Kayali H. The effect of iron and copper as an essential nutrient on mitochondrial electron transport system and lipid peroxidation in Trichoderma harzianum. Appl Biochem Biotechnol. 2013;170:1665–75.
[5] Li QF, Ding XQ, Kang YJ. Copper promotion of angiogenesis in isolated rat aortic ring: role of vascular endothelial growth factor. J Nutr Biochem. 2014;25:44–9.
[6] Wang Z, Zhao Y, Zhao Y, et al. Exosomes secreted by macrophages upon copper ion stimulation can promote angiogenesis. Mater Sci Eng C Mater Biol Appl 2021;123:111981.
[7] Mitra S, Keswani T, Dey M, et al. Copper-induced immunotoxicity involves cell cycle arrest and cell death in the spleen and thymus. Toxicology 2012;293:78–88.
[8] Sogkas G, Atschekzei F, Adriawan IR, et al. Cellular and molecular mechanisms breaking immune tolerance in inborn errors of immunity. Cell Mol Immunol. 2021;18:1122–40.
[9] Huang AF, Su LC, Jia H, et al. No association of single nucleotide polymorphisms within H19 and HOX transcript antisense RNA (HOTAIR) with genetic susceptibility to systemic lupus erythematosus, rheumatoid arthritis, and primary Sjögren's syndrome in a Chinese Han population. Clin Rheumatol. 2017;36:2447–53.
[10] Li N, Li Y, Hu J, et al. A link between mitochondrial dysfunction and the immune microenvironment of salivary glands in primary Sjögren's syndrome. Front Immunol. 2022;13:845209.
[11] Amna T, Van Ba H, Vaseem M, et al. Apoptosis induced by copper oxide quantum dots in cultured C2C12 cells via caspase 3 and caspase 7: a study on cytotoxicity assessment. Appl Microbiol Biotechnol. 2013;97:5545–53.
[12] Santos S, Silva AM, Matos M, et al. Copper induced apoptosis in Caco-2 and Hep-G2 cells: expression of caspases 3, 8 and 9, AIF and p53. Comp Biochem Physiol C Toxicol Pharmacol. 2016;185-186:138–46.
[13] Reina S, Sterin-Borda L, Borda E. Anti-M peptide IgG from Sjögren's syndrome triggers apoptosis in A253 cells. Cell Immunol. 2012;275:33–41.
[14] Tsvetkov P, Coy S, Petrova B, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. Science 2022;375:1254–61.
[15] Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44:W90–97.
[16] Lin Y, Yao X, Yan M, et al. Integrated analysis of transcriptomics to identify hub genes in primary Sjogren's syndrome. Oral Dis. 2021;12:697157.
[17] Hou X, Hong X, Ou M, et al. Analysis of gene expression and TCR/B Cell receptor profiling of immune cells in primary Sjogren's syndrome by single-cell sequencing. J Immunol. 2022;209:238–49.
[18] Dolcino M, Tinazzi E, Vitali C, et al. Long non-coding RNAs modulate Sjogren's syndrome associated gene expression and are involved in the pathogenesis of the disease. J Clin Med 2019;8:1349.
[19] Dong Y, Ming B, Gao R, et al. The IL-33/ST2 Axis promotes primary Sjogren's syndrome by enhancing salivary epithelial cell activation and type 1 immune response. J Immunol. 2022;208:2652–62.
[20] Xanthou G, Tapinos NI, Polihronis M, et al. CD4 cytotoxic and dendritic cells in the immunopathologic lesion of Sjögren's syndrome. Clin Exp Immunol. 1999;118:154–63.
[21] Bave U, Nordmark G, Lovgren T, et al. Activation of the type I interferon system in primary Sjögren's syndrome: a possible etiopathogenic mechanism. Arthritis Rheum. 2005;52:1185–95.
[22] Choi W, Li Z, Oh HJ, et al. Expression of CCR5 and its ligands CCL3, -4, and -5 in the tear film and ocular surface of patients with dry eye disease. Curr Eye Res. 2012;37:12–7.
[23] Ozaki Y, Ito T, Son Y, et al. Decrease of blood dendritic cells and increase of tissue-infiltrating dendritic cells are involved in the induction of Sjögren's syndrome but not in the maintenance. Clin Exp Immunol. 2010;159:315–26.
[24] Lopes AP, van Roon JAG, Blokland SLM, et al. MicroRNA-130a contributes to Type-2 classical DC-activation in Sjögren's syndrome by targeting mitogen- and stress-activated protein kinase-1. Front Immunol. 2019;10:1335.
[25] Ferlazzo G, Tsang ML, Moretta L, et al. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. J Exp Med. 2002;195:343–51.
[26] Mills KH. Induction, function and regulation of IL-17-producing T cells. Eur J Immunol. 2008;38:2636–49.
[27] Gliozzi M, Greenwell-Wild T, Jin W, et al. A link between interferon and augmented plasmin generation in exocrine gland damage in Sjögren's syndrome. J Autoimmun. 2013;40:122–33.
[28] Rizzo C, Grasso G, Destro Castaniti GM, et al. Primary Sjögren's syndrome: focus on innate immune cells and inflammation. Vaccines (Basel) 2020;8:272.
[29] Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol. 2007;8:191–7.
[30] Li B, Xing Y, Gan Y, et al. Labial gland-derived mesenchymal stem cells and their exosomes ameliorate murine Sjögren's syndrome by modulating the balance of Treg and Th17 cells. Stem Cell Res Ther. 2021;12:478.
[31] Furuzawa-Carballeda J, Hernandez-Molina G, Lima G, et al. Peripheral regulatory cells immunophenotyping in primary Sjögren's syndrome: a cross-sectional study. Arthritis Res Ther. 2013;15:R68.
[32] Alunno A, Petrillo MG, Nocentini G, et al. Characterization of a new regulatory CD4+ T cell subset in primary Sjögren's syndrome. Rheumatology (Oxford) 2013;52:1387–96.
[33] Jirsova K, Seidler Stangova P, Palos M, et al. Aberrant HLA-DR expression in the conjunctival epithelium after autologous serum treatment in patients with graft-versus-host disease or Sjögren's syndrome. PLoS One. 2020;15:e0231473.
[34] Shah NR, Noll BD, Stevens CB, et al. Biosemantics guided gene expression profiling of Sjögren's syndrome: a comparative analysis with systemic lupus erythematosus and rheumatoid arthritis. Arthritis Res Ther. 2017;19:192.
[35] Yao Q, Song Z, Wang B, et al. Identifying key genes and functionally enriched pathways in Sjögren's syndrome by weighted gene co-expression network analysis. Front Genet. 2019;10:1142.
[36] Fang TJ, Li RN, Lin YZ, et al. Association of F11R polymorphisms and gene expression with primary Sjögren's syndrome patients. Int J Rheum Dis 2021;24:681–6.
[37] Chen W, Sun X, Zhan L, et al. Conditional knockout of Pdha1 in mouse hippocampus impairs cognitive function: the possible involvement of lactate. Front Neurosci. 2021;15:767560.
[38] Liu L, Cao J, Zhao J, et al. PDHA1 gene knockout in human esophageal squamous cancer cells resulted in greater Warburg effect and aggressive features in vitro and in vivo. Onco Targets Ther 2019;12:9899–913.
[39] Wu S, Wang W, Le Q. All-trans retinoic acid regulates the balance of Treg-Th17 cells through ERK and P38 signaling pathway. Iran J Immunol. 2019;16:1–10.
[40] Sun X, Xiao Y, Zeng Z, et al. All-Trans retinoic acid induces CD4+CD25+FOXP3+ Regulatory T cells by increasing FOXP3 demethylation in systemic sclerosis CD4+ T Cells. J Immunol Res 2018;2018:8658156.
[41] Parastouei K, Mirshafiey A, Eshraghian MR, et al. The effect of 1, 25(OH)2 D3 (calcitriol) alone and in combination with all-trans retinoic acid on ROR-gammat, IL-17, TGF-beta, and FOXP3 gene expression in experimental autoimmune encephalomyelitis. Nutr Neurosci. 2018;21:210–8.

[42] Xie X, Mu L, Yao X, et al. ATRA alters humoral responses associated with amelioration of EAMG symptoms by balancing Tfh/Tfr helper cell profiles. Clin Immunol. 2013;148:162–76.
[43] Bidad K, Salehi E, Oraei M, et al. Effect of all-trans retinoic acid (ATRA) on viability, proliferation, activation and lineage-specific transcription factors of CD4+ T cells. Iran J Allergy Asthma Immunol. 2011;10:243–9.
[44] Park JS, Moon SJ, Lim MA, et al. Retinoic acid receptor-related receptor alpha ameliorates autoimmune arthritis via inhibiting of Th17 cells and osteoclastogenesis. Front Immunol. 2019;10:2270.
[45] Hagenstein J, Melderis S, Nosko A, et al. A novel role for IL-6 receptor classic signaling: induction of RORgammat(+)Foxp3(+) Tregs with enhanced suppressive capacity. J Am Soc Nephrol. 2019;30:1439–53.
[46] Yang J, Xu L. Elevated IL-23R expression and Foxp3+Rorgt+ cells in intestinal mucosa during acute and chronic colitis. Med Sci Monit. 2016;22:2785–92.
[47] Martinez-Blanco M, Lozano-Ojalvo D, Perez-Rodriguez L, et al. Retinoic acid induces functionally suppressive foxp3(+)RORgammat(+) T cells in vitro. Front Immunol. 2021;12:675733.
[48] Ball JA, Clear A, Aries J, et al. Retinoic acid-responsive CD8 effector T cells are selectively increased in IL-23-rich tissue in gastrointestinal GVHD. Blood 2021;137:702–17.
[49] Rafe T, Shawon PA, Salem L, et al. Preventive role of resveratrol against inflammatory cytokines and related diseases. Curr Pharm Des. 2019;25:1345–71.
[50] Inoue H, Kishimoto A, Ushikoshi-Nakayama R, et al. Resveratrol improves salivary dysfunction in a non-obese diabetic (NOD) mouse model of Sjögren's syndrome. J Clin Biochem Nutr. 2016;59:107–12.
[51] Wang J, Zhang Z, Fang A, et al. Resveratrol attenuates inflammatory bowel disease in mice by regulating SUMO1. Biol Pharm Bull. 2020;43:450–7.