Uncovering the Therapeutic Target and Molecular Mechanism of Upadacitinib on Sjogren's Syndrome

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Sage

ABSTRACT

OBJECTIVE: Upadacitinib, a selective Janus associated kinase 1 (JAK-1) inhibitor, can be prescribed particularly for the clinical treatment with Crohn's disease or rheumatoid arthritis. It is clinically observed that upadacitinib has been found with potential therapeutic effectiveness on Sjogren's syndrome (SS). However, the anti-SS targets and mechanisms involved in upadacitinib treatment remain uninvestigated.

MATERIALS AND METHODS: Thus, this study was designed to identify therapeutic targets and mechanisms of upadacitinib for treating SS through conducting network pharmacology and molecular docking analyses.

RESULTS: In total, we identified 298 upadacitinib-related target genes, 1339 SS-related targets before collecting 56 overlapped target genes and 12 hub target genes. Upadacitinib largely exerted the critical biological processes including regulation of microenvironment homeostasis, inflammatory response, and cell apoptosis, and largely acted on pivotal molecular mechanisms including hypoxia-inducible factor 1 (HIF-1) signaling pathway, apoptosis pathway, phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway, or Th17 cell differentiation pathway. Molecular docking data suggested that upadacitinib exhibited the high affinities with signal transducer and activator of transcription 3 (STAT3), HIF1A, poly(ADP-ribose) polymerase 1 (PARP1) target proteins, in which the structural interactions between upadacitinib and STAT3, HIF1A, PARP1 showed potential therapeutic activities against SS.

CONCLUSION: In conclusion, upadacitinib possesses the bright anti-inflammatory and anti-apoptotic activities on SS, and this study can provide a theoretical basis for clinical therapy of SS using upadacitinib.

KEYWORDS: Upadacitinib, Sjogren's syndrome, inflammation, apoptosis, therapeutic genes

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Introduction

Sjogren's syndrome (SS), a kind of systemic autoimmune disease, is derived from abnormally altered structure and function in exocrine glands, including the lacrimal and salivary glands.[1] SS-pathological symptoms can be found in different organs, and the functions from lacrimal and salivary glands are evidently impacted and then lead to severe dryness in mouth and eye, thus mainly showing the clinical manifestations as xerophthalmia and xerostomia.[2] The morbidity ratio of SS ranges from 0.1% to 0.3% in the world, in which SS ranks the second-highest disorder in rheumatic immune diseases and results in high global burden.[3] It is reported that SS patients can confront bad quality of life, and this disease can ultimately aggravate the physiological and psychological burdens to the patients.[4] To date, the accurate pathogenesis involved in SS is still unclear. Mounting evidences show that T and B cells actively activate in induction of systemic complications in SS from autoantibody generation to cytokine release.[5] It is reported that innate and adaptive immune responses are responsible for the occurrence and progression of SS.[6] In clinical medication, some immunomodulatory drugs, such as hydroxychloroquine, azathioprine, are prescribed for the treatment with SS.[7] However, the therapeutic effectiveness using immunomodulatory medicines are unmet need and SS patients may occur in concomitant side-effects, including hypocalcemia, vomiting, nausea, and myasthenia.[8] Upadacitinib refers to a selective, small molecular JAK inhibitor that exhibits the high targeting of JAK1.[9] A multi-center, double-blind, randomized trial suggests that upadacitinib presents marked efficacy and safety characteristics for patients with moderate-to-severe ulcerative colitis.[10] Another randomized clinical trial shows that upadacitinib exerts better efficacy and safety features on moderate-to-severe atopic dermatitis patients when compared to dupilumab treatment.[11] In addition, upadacitinib represents a substitute opinion for the treatment with psoriatic arthritis patients, exhibiting certain beneficial features.[12] Upadacitinib has showed the specific and effective features to treat rheumatoid arthritis in comparison with other medicines, including Tofacitinib, Baricitinib.[13] Interestingly, upadacitinib is clinically observed with potential effects for treating SS. Based on clinical and experimental reports, JAK-targeted inhibitors, such as upadacitinib, may be widely used to treat connective

tissue diseases (CTDs) including SS through lessening cytokine release and inhibiting inflammatory injury.[14] Although the effectiveness in clinical observation is prominent, the anti-SS biotargets and therapeutic mechanisms of upadacitinib remain unknown. Therefore, the comprehensive efficacy of upadacitinib against SS is worth further exploration. Network pharmacology is a favoring toolkit for drug discovery by using comprehensive interdisciplinary disciplines, including systemic pharmacology, bioinformatics analysis based on databank paradigms.[15] Molecular docking approach can be used for identifying the optimal interactions between ligand and protein at molecular level for biomolecular visualization and analysis.[16] In present research, network pharmacology strategy was used to screen and identify the anti-SS target genes associated with upadacitinib, and to unveil the therapeutic mechanisms of upadacitinib against SS. Meanwhile, molecular docking technology was used to testify the ligand-protein recognition process, structure-activity relationships in upadacitinib and key proteins. This research aimed to provide a useful insight into the future clinical application of upadacitinib for the treatment with SS through using computational integrated analysis.

Materials and Methods

Potential therapeutic targets in upadacitinib on SS

Availably, upadacitinib-linked targets were searched and sorted from the Swiss Target Prediction (www.swisstargetprediction.ch) and Super-pred (https://prediction.charite.de/subpages/target_prediction.php) databases, and the selective targets were normalized using UniProt (https://www.uniprot.org) tool by using the setting of "*Homo sapiens*." SS-related targets were downloaded and obtained from the GeneCards (https://www.genecards.org/) database, Online Mendelian Inheritance in Man (OMIM, https://omim.org/search/advanced/geneMap) database and DisGeNET (http://www.disgenet.org/) database by using the keyword of "Sjogren's Syndrome." The overlapped protein targets were acquired by using Venny platform (https://bioinfogp.cnb.csic.es/tools/venny/index.html). These overlapped proteins were considered as potential therapeutic targets in upadacitinib on SS.

Protein-protein interaction (PPI) network generation

The PPI network diagram was built by using the Retrieval of Interacting Genes (STRING; https://string-db.org/) search tool. The Cytoscape (https://cytoscape.org/) software was utilized for the numeration of degree centrality of each interaction, and the top key targets were discovered for further revelation. Thus, a PPI network diagram involved in key targets was produced, and the nodes denoted to genes while edges expressed to gene-gene relationships, respectively.

Gene Ontology (GO) function and Kyoto Encyclopedia Gene and Genomes (KEGG) pathway enrichment analyses

All key targets were imported to Cytoscape (https://cytoscape.org/) software, and enrichment analysis of GO-based biological process and KEGG-based pathway profile were successively performed following the adjusted *P*-value less than .05. Target genes was used for the GO analysis with the main functional categories including biological processes (BPs), cellular components (CCs), molecular functions (MFs). KEGG analysis with the top signaling pathways was depicted. The GO and KEGG enrichment data were uploaded into SangerBox (http://vip.sangerbox.com/) analysis platform for diagrammatic visualization.

Molecular docking technology

The 2-dimensional structure of upadacitinib was downloaded from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database and then was transformed to 3-dimensional structure. After computation of energy standardization, these analytical structure files were saved as mol2 format. The 3-dimensional structures of key target proteins in STAT3, HIF1A, PARP1 were collected from the structural bioinformatics Protein Data Bank (RCSB PDB, http://www.rcsb.org/pdb/) database. The AutoDock Tools (https://autodock.scripps.edu/) software was used to change file format to another pdbqt format. The active affinities between ligands and target proteins were calculated as binding energies through using AutoDock Vina (https://vina.scripps.edu/) software, and the optimal data were defined as binding energy to target receptors when less than −5 kcal/mol. And the PyMOL (https://www.pymol.org/) software was conducted for expansion of ligand docking and binding site.[16]

Results

Collection of various targets

After removing discrepancy targets, the 298 drug-associated targets were obtained in upadacitinib, and 1339 disease-associated targets were collected in SS. Both drug and disease targets were transferred to the Venn database for further assay, and then total 56 overlapped target genes were identified finally (Figure 1A). The upadacitinib-SS-target network involved in 56 targets was built, characterized with interlaced nodes and edges for functional significances (Figure 1B).

Construction of PPI network and identification of key targets

The 56 overlapped target genes were analyzed again by using String tool, and thus a PPI network diagram was established

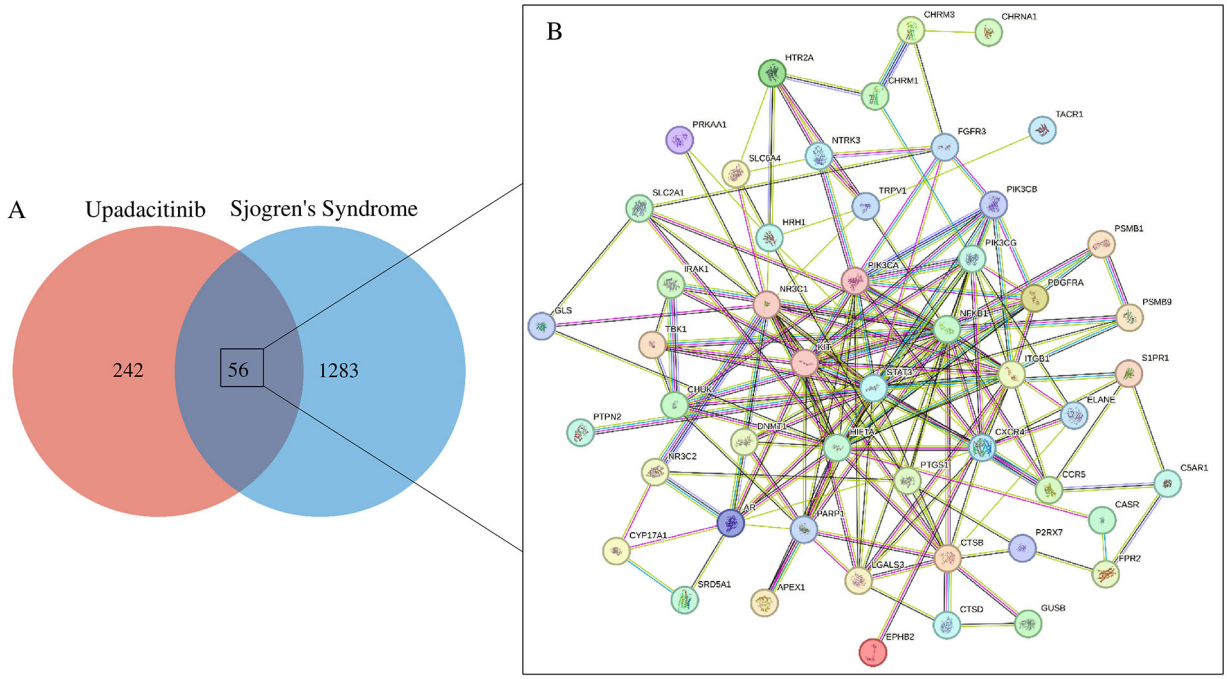

Figure 1. The 56 overlapped target genes might be potential anti-SS targets of upadacitinib. (A) A Venn diagram reflecting representational target genes in upadacitinib, SS. (B) The PPI network illustration containing all overlapped target genes for correlative visualization.

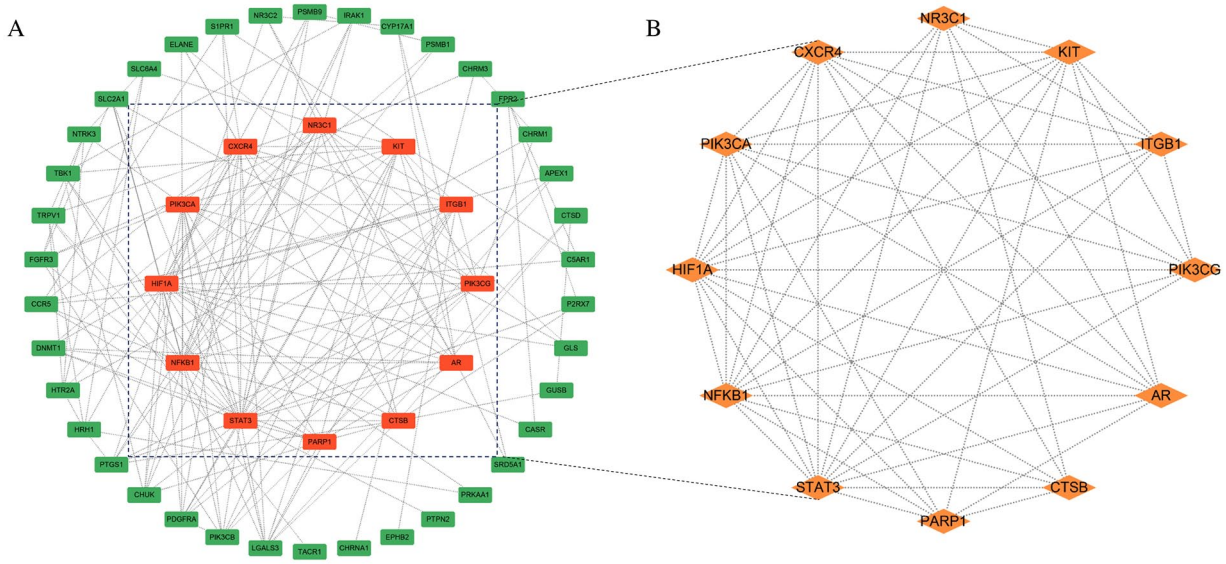

Figure 2. The potential therapeutic targets of upadacitinib in the treatment with SS. (A) The drug-target network of upadacitinib treating SS. Green nodes represented the overlapped targets, and russet nodes represented the key targets. (B) Key targets identified by algorithmic values showing in a correlative network.

connectedly (Figure 2A). After algorithmic analysis, 12 target genes were screened, and these genes were identified as key targets that might act on therapeutic benefits of upadacitinib against SS. The key targets including signal transducer and activator of transcription 3 (STAT3), nuclear factor kappa B subunit 1 (NFKB1), hypoxia-inducible factor 1 (HIF-1), phosphoinositol-3-kinase catalytic subunit alpha (PIK3 CA), C-X-C motif chemokine receptor 4 (CXCR4), glucocorticoid receptor gene (NR3C1), c-kit (KIT), integrin beta1 (ITGB1), androgen receptor (AR), phosphoinositol-3-kinase catalytic subunit gamma (PIK3CG), cathepsin B (CTSB) and poly(ADP-ribose) polymerase 1 (PARP1) were highlighted in Figure 2B.

GO and KEGG enrichment findings

In order to uncover upadacitinib treating SS through biological functions, GO enrichment analysis (adjusted $P < .05$) was completed with key target genes on the processes of BPs, CCs, and MFs. The top GO items were shown in Figure 3A. The

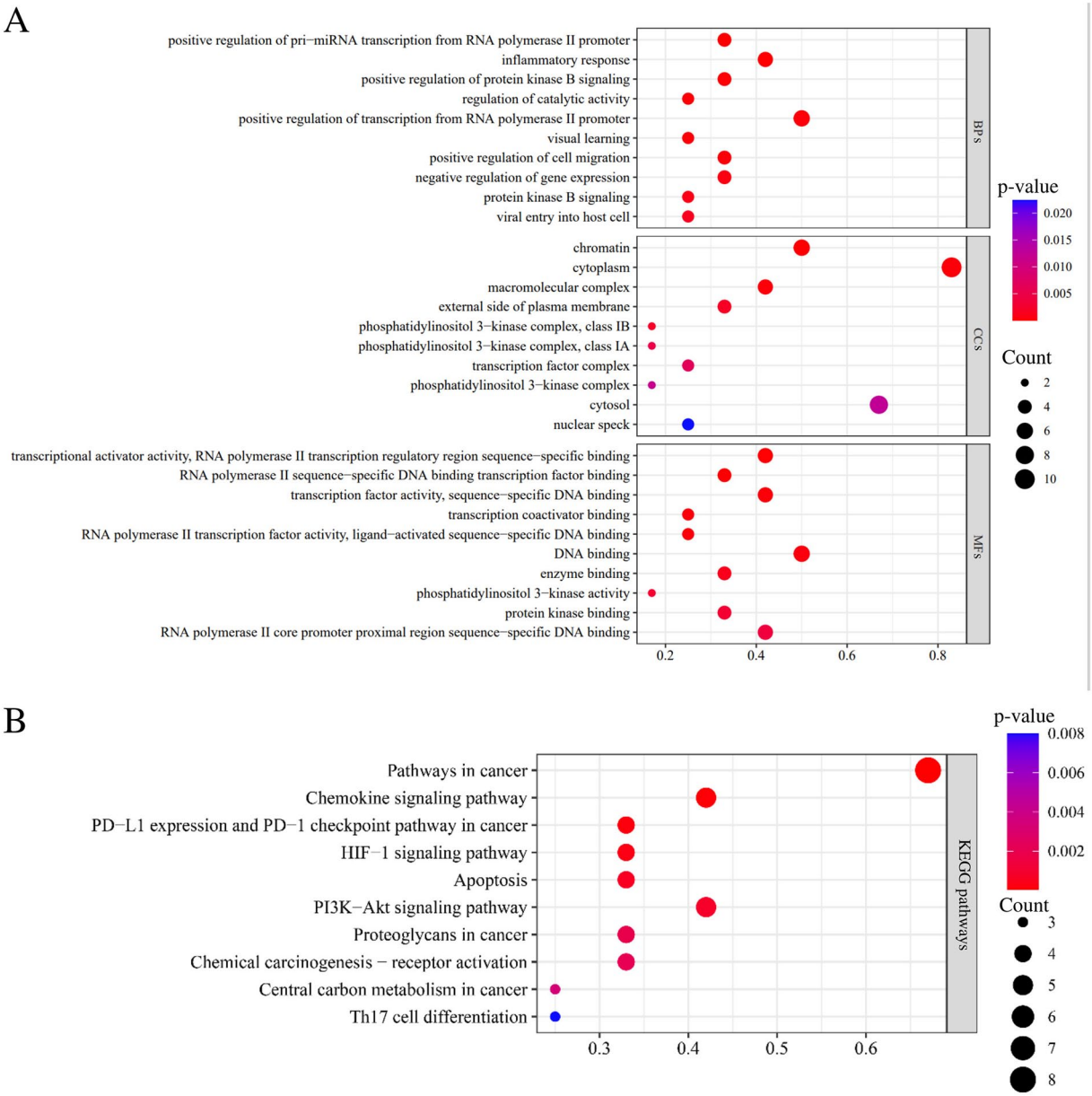

Figure 3. (A) GO enrichment analysis of key targets in upadacitinib treating SS in main categories of BPs, CCs, and MFs. Larger dark color spots represented higher importance to treat SS. (B) KEGG enrichment analysis of key targets in upadacitinib treating SS. The color of bubble represented the significance of enrichments.

bubble size was larger, and then the enrichment score was higher. As shown in BP items, major BPs were related to positive regulation of pri-miRNA transcription, inflammatory response, positive regulation of cell migration or negative regulation of gene expression. As shown in CC items, major CCs were related to macromolecular complex, phosphatidylinositol 3-kinase complex, or transcription factor complex. As shown in MF items, major MFs were related to RNA polymerase II sequence-specific DNA binding transcription factor binding, transcription factor activity, sequence-specific DNA binding, transcription coactivator binding, enzyme binding or protein kinase binding. These key targets were enriched and showed in optimal pathways (adjusted $P < .05$). A total of 10 top molecular pathways were identified, and major items were mainly targeted to SS, such as HIF-1 signaling pathway, apoptosis-related pathway, PI3K–Akt signaling pathway, or Th17 cell differentiation-related pathway. In addition, it was concluded that network pharmacology characteristics with multiple functions and multiple pathways in upadacitinib treating SS were highlighted in Figure 3B.

Binding affinity characteristics in molecular docking technology

Molecule docking analysis was performed for characterizing upadacitinib docking with target proteins in SS. The 2-dimensional structure of upadacitinib and the crystal structures of key proteins including STAT3, HIF1A, PARP1 were acquired

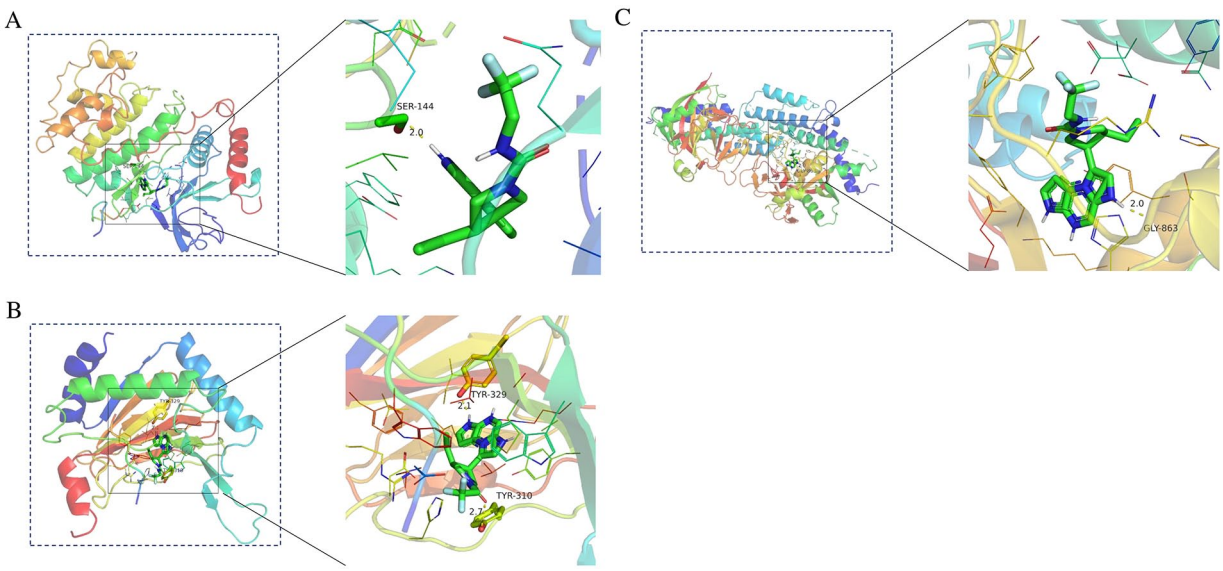

Figure 4. Molecular docking models of upadacitinib binding to key target proteins. (A) STAT3-5AX3: upadacitinib; (B) HIF1A-3HQU: upadacitinib; (C) PARP1-7KK4: upadacitinib.

before biological docking. The binding energy scores were upadacitinib and STAT3 (−6.9 kcal/mol), upadacitinib and HIF1A (−8.3 kcal/mol), and upadacitinib and PARP1 (−8.8 kcal/mol), indicating the powerful binding affinities. As shown in Figure 4, there was an effective hydrogen bond between upadacitinib and STAT3-5AX3, including SER-144 (2.0 Å) (Figure 4A), upadacitinib and HIF1A-3HQU, including TYR-329 (2.1 Å), TYR-310 (2.7 Å; Figure 4B), upadacitinib and PARP1-7KK4, including GLY-863 (2.0 Å; Figure 4C).

Discussion

Although SS can be observed in clinical experience, its pathomechanism is still indistinct roundly. The pathogenesis of SS is complicated, such as innate immune dysfunction, microenvironment disarray, inflammatory infiltration, epithelial cell destruction in glands.[17] Current clinical pharmacotherapy on SS is still limited, and the candidate medication should be explored and developed for the potential clinical treatment to SS. Certain clinical observations reported by clinician show that upadacitinib, an oral selective inhibitor of JAK-1, can relieve the symptoms in SS. Although the therapeutic effectiveness is found, the pharmacological target and molecular mechanism of upadacitinib treating SS remain unstudied. In this research, a systematic bioinformatics using network pharmacology analysis was utilized to uncover anti-SS therapeutic target and mechanism of upadacitinib, thus providing theoretical support for futural clinical application. The common targets in upadacitinib and SS were screened firstly, and then a total of 56 overlapped target genes using the PPI analysis was obtained, exhibiting that upadacitinib could interact on multiple pharmacological targets for potentially treating SS. All key targets were further identified, including STAT3, NFKB1, HIF-1, PIK3CA, CXCR4, NR3C1, KIT, ITGB1, AR, PIK3CG, CTSB, and PARP1. And GO enrichment analysis for therapeutic targets suggested that pri-miRNA transcription, inflammatory response, positive regulation of cell migration or negative regulation of gene expression were some key terms in biological processes associated with upadacitinib treating SS. MicroRNAs (miRNAs) is tightly related to disease development and microenvironment function through regulating targeting gene expressions, including autoimmune SS.[18] Chronic inflammatory response may be one of the pathological causes of SS, and the dysfunctional mitochondria in salivary glands is interrelated additionally.[19] These data indicated that upadacitinib might effectively modulate immune microenvironment functions to restrain inflammatory reaction for treating SS. As highlighted by KEGG enrichment findings, the anti-SS targets of upadacitinib were mostly enriched in HIF-1 signaling pathway, apoptosis-related pathway, PI3K–Akt signaling pathway, or Th17 cell differentiation-related pathway. Apoptosis in SS patient's exocrine gland cells is closely triggered through modulation of pro-apoptotic signaling pathways, cellular survival molecules and cytotoxic mediators.[20] PI3K-Akt activities have been found with Toll-like receptor 3-caused cell apoptosis in salivary gland epithelial cells, and lymphocyte infiltration is medically detected in SS patients when PI3K pathway activated.[21] Thereby, direct inhibition of cell apoptosis, inflammatory reaction and effective enhancement of immunomicroenvironment in gland tissues/cells may be potential therapeutic activities for upadacitinib treating SS. In further molecular docking analysis, the computational results indicated the powerful binding affinities between upadacitinib and key proteins, including STAT3, HIF1A, PARP1. In primary SS

patients, STAT3 phosphorylation is positively induced in CD4(+) lymphocytes due to abnormal STAT3 activation in Tfh cells for overproducing CD4(+) cells.[22] It is clinically found that HIF1A gene polymorphisms is related to the development of primary SS, in which the HIF1A Pro582Ser T allele genotype acts as potential genetic factors related to SS.[23] Notably, the elevated expression of apoptosis-related PARP-1 activity in impaired salivary gland samples from SS patients is shown to be associated with the phosphorylation of c-Jun N-terminal kinase.[24] Thus, these STAT3, HIF1A, PARP1 key genes identified may be one of potential anti-SS targets in upadacitinib. However, this study also existed certain actual limitations. For instance, current research was expected that more experiments in vivo or in vitro should be performed to validate these preclinical conclusions.

Conclusion

Taken together, the results from computational research highlight that upadacitinib might largely maintain microenvironment homeostasis, inhibit inflammatory response and cell apoptosis to function the comprehensive actions on treating SS. Meantime, STAT3, HIF1A, and PARP1 may be potential anti-SS targets of upadacitinib. These data indicate that upadacitinib exhibits the promising characteristics through multi-targets, and multi-pathways to treat SS, implying that upadacitinib may be a potential drug for clinically treating SS.

Author contributions

Y. Y. and Y. L.: Conceptualization, Methodology, Project administration, Writing manuscript; X. L., Y. Z., W. H. and J. Z.: Software, Formal analysis, Resources, Data Curation, Visualization.

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