Network pharmacology and data analysis method to explore the traditional Chinese medicine regulates ferroptosis key genes in the occurrence and prognosis of lupus nephritis

Ai-Tao Lin⁠¹, Jin-Yu Wu⁠²*, Zhi-Ying Zhang⁠³

¹Ai-Tao Lin and Jin-Yu Wu are the co-first authors of this paper.
²Graduate school, Guangxi University of Traditional Chinese Medicine, Nanning 530001, China. ³Department of Rheumatology, The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning 530023, China.

Corresponding to: Jin-Yu Wu, Department of Rheumatology, The First Affiliated Hospital of Guangxi University of Chinese Medicine, No. 13, Wuhe Avenue, Qingxiu District, Nanning 530023, China. E-mail: wujinyu0109@sina.com.

Abstract

Purpose: To explore the traditional Chinese medicine (TCM) regulates ferroptosis key genes in the occurrence and development of lupus nephritis (LN) based on biological information database. Patients and methods: Ferroptosis related genes were identified based on FerrDb database and literature retrieval. Used the OMIM, Gene Cards, Drug Bank to obtain the targets of LN. Cytoscape 3.8.2 software and STRING database were used to analyze protein-protein interaction (PPI) network. Metacapse software and Weishengxin were used to analyze the gene ontology (GO) classification and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis. UniProt Database and Traditional Chinese Systems Pharmacology Database and Analysis Platform analysis platform were used to obtain the data table of key TCM and related targets. Cytoscape 3.8.2 software was used to analyze the PPI network. Results: A total of 401 ferroptosis-related genes, 361 LN related genes and 21 “Ferroptosis-LN” intersection genes were obtained. Ferroptosis in the occurrence and prognosis of LN mainly involved the inflammatory response, cell activation, positive regulation of chemokine production and it was mainly involved in necroptosis, inflammatory bowel disease, ferroptosis and other pathways. A total of 412 TCMs containing key genes of “Ferroptosis-LN” were acquired. The most key genes were contained in Mahuang, Gehua, Baiguo, Chuanniuxi, Jinyinhu. 15 key genes of “TCM-LN” were obtained. 5 ferroptosis-related key genes in LN regulated by TCM were obtained, which were IL1β, TLR4, IFNG, STAT3 and HMOX1. Conclusion: TCM, such as Mahuang, Gehua, Baiguo, Chuanniuxi, Jinyinhu, may affect the occurrence and development of LN through the key ferroptosis genes, such as IL1B, TLR4, IFNG, STAT3 and HMOX1.

Keywords: lupus nephritis; network pharmacology; data mining; ferroptosis; traditional Chinese medicine
Introduction

Systemic Lupus Erythematosus (SLE) represents an autoimmune connective tissue ailment characterized by heightened activity within T and B lymphocytes, which lead to the production of a substantial volume of autoantibodies [1–3]. Lupus nephritis (LN), encompassing approximately 60% of diagnosed SLE cases, is recognized for inducing severe harm, posing treatment challenges, exhibiting frequent relapses, and displaying a discouraging prognosis. LN places a substantial burden on public health [4, 5]. Ferroptosis, primarily induced by the accumulation of glutathione peroxidase 4 (GPX4) and lipid reactive oxygen species (ROS), is a form of cell demise. Prior investigations have established that ferroptosis triggers the innate immune and inflammatory responses, subsequently releasing inflammatory mediators, including IL1β and IL18, both associated with inflammatory disorders [6]. The MRL-Faslpr mouse study revealed that type I interferon and IgG diminish GPX4 expression, a pivotal contributor to ferroptosis [7].

Traditional Chinese medicine (TCM) has demonstrated noteworthy clinical effectiveness in addressing LN. Outcomes from preliminary animal experiments conducted by our research group have indicated that Pannotoginseng saponins, a component of TCM, have the potential to obstruct renal inflammation by impeding the TGF-β1/Smads and TGF-β1/P38MAPK signaling pathways. This leads to the downregulation of integrin ligases, hindrance of renal tubular epithelial cell transdifferentiation, and inhibition of CRP-mediated inflammatory reactions [8–10]. Leveraging network pharmacology techniques and data analysis, this study delves into the critical ferroptosis-associated genes influenced by TCM in the initiation and prognostication of LN [11]. These findings introduce novel perspectives for treating LN with TCM.

Material and methods

Acquisition of ferroptosis-related genes

Ferroptosis-related genes were acquired through a search of the FerrDb (http://www.zhounan.org/ferrdb) database and the literature, with duplicate genes subsequently eliminated.

Acquisition of LN-related genes

Using “lupus nephritis” as the search term via Drug Bank (https://go.drugbank.com/), OMIM (https://www.omim.org/), Gene Cards (https://www.genecards.org/). The databases were searched for LN-related genes, which were removed duplicate genes.

Acquisition of “ferroptosis-LN” intersection genes and construction of protein-protein interaction (PPI) networks

The “ferroptosis-LN” intersection genes were obtained using VENNY 2.1.0 (https://bioinfopg.cnb.csic.es/tools/venny/). These intersection genes were then fed into the STRING database, with the species designated as “Homo sapiens”, to retrieve the PPI network file. These files were subsequently imported into Cytoscape 3.8.2 software to create a visual representation, constructing the “ferroptosis-LN” PPI network component diagram.

Gene ontology (GO) analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

Utilize the Metacape database (https://metacape.org/) to conduct an analysis of the Biological Process, Molecular Function, and Cellular Component attributes of the “ferroptosis-LN” co-protein for GO analysis, as well as for performing KEGG enrichment pathway analysis. Employ microbiology software (http://www.bioinformatics.com.cn/) to generate visual representations of KEGG pathway enrichment analyses using bubble charts.

Acquisition of key TCMs from the “ferroptosis-LN” intersection genes

The “ferroptosis-LN” intersection genes obtained in Section 2.3 were introduced into the UniProt (https://www.uniprot.org/) database, with the species specified as “Homo sapiens”, thereby acquiring the complete protein names. Subsequently, the comprehensive protein names were integrated into the Traditional Chinese Systems Pharmacology Database and Analysis Platform (TCMSP) database to retrieve the principal TCMs from the set of “ferroptosis-LN” intersection genes.

“TCM-target” network construction and “TCM-LN” PPI network construction

The TCM retrieved in Section 2.3 were inputted into the TCMSP database, with screening criteria set at Oral Bioavailability ≥ 30% and Drug-likeness ≥ 0.18, in order to acquire their active components. Subsequently, the corresponding targets for these active components were identified. Following the exclusion of non-standard and non-human targets, the principal TCM target database for the “ferroptosis-LN” intersection genes was established. Cytoscape 3.8.2 software was utilised to formulate the “TCM-target” network. The convergence of TCM target genes and LN target genes was extracted using VENNY 2.1.0 software, and this outcome was then visually represented through the STRING database and Cytoscape 3.8.2 software.

Acquisition of key genes for the regulation of ferroptosis associated with LN by TCM

The overlap between the key genes of “ferroptosis-LN” and “TCM-LN” was derived using VENNY 2.1.0 software, thereby extracting the key genes involved in TCM’s regulation of ferroptosis-associated LN.

Results

Acquisition of ferroptosis-related genes

A sum of 268, 282, and 3 ferroptosis-related genes were procured from the “Driver”, “Suppressor”, and “Marker” modules within the FerrDb database, respectively. Following the exclusion of duplicate genes, a total of 401 genes were obtained, thereby constituting a database of genes linked to ferroptosis.

Acquisition of LN-related genes

A combined total of 1330 genes related to LN were sourced, encompassing 12 genes from OMIM, 1275 genes from Gene Cards, and 42 genes from Drug Bank. Out of these, the uppermost 320 genes with elevated scores were culled from the Gene Cards database. Following the removal of duplicated genes, a compilation of 361 genes was procured, thereby establishing a comprehensive LN-related gene repository.

Acquisition of “ferroptosis-LN” intersection genes

The ferroptosis-related genes and LN-related genes were inputted into VENNY 2.1.0, revealing the overlap of “ferroptosis-LN” genes as depicted in Figure 1.
PPI network of “ferroptosis-LN” intersection genes

Figure 2 illustrates the establishment of the PPI network comprising 19 shared genes that interact within the “ferroptosis-LN” context, with 2 irrelevant genes excluded. Gene interrelationships were denoted using “edges” and “nodes”, yielding a graph with 19 nodes and 87 edges. The graph showcased an average node degree of 9.16, an average local clustering coefficient of 0.818, and a PPI enrichment value below 10e-16. Elaborated information about these 19 intersecting genes can be found in Table 1.

GO analysis and KEGG pathway enrichment analysis

The Metacape database yielded 24 outcomes from GO functional enrichment analysis, encompassing 19 results for Biological Processes, primarily associated with inflammatory responses, cellular activation, positive regulation of chemokine production, negative regulation of immune system processes, and regulation of defense responses; 3 results for Cellular Components, mainly linked to receptor complexes, secretory granule endosomes, and lysis vesicles; and 2 results for Molecular Function, predominantly involving cytokine activity and transcription factor binding. Refer to Figure 3 for comprehensive details. Additionally, 9 pathways emerged from the KEGG pathway enrichment analysis, with the primary pathways that have been previously identified as pertinent to LN encompassing Necrotosis, Inflammatory bowel disease, Pathways in cancer, HIF-1 signaling pathway, and the Ferroptosis pathway. For an in-depth understanding, consult Figure 4.

Table 1 Commonly related genes information of Ferroptosis and LN

<table>
<thead>
<tr>
<th>Numbe</th>
<th>Gene Name</th>
<th>Full name of the gene</th>
<th>Degree value</th>
<th>Node Tightness</th>
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<td>Toll-like receptor 4</td>
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<td>0.8182</td>
</tr>
<tr>
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<td>Signal transduction and transcriptional activator 3</td>
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<td>0.8182</td>
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<td>IFNG</td>
<td>Interferon</td>
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<td>0.7826</td>
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<td>Mammalian rapamycin</td>
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<td>Interferon 1</td>
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<td>TNFAIP3</td>
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<td>Autophagy gene 5</td>
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<td>Glutathione S-transferase M1</td>
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</table>

LN, lupus nephritis.
Acquisition of key TCMs from the “ferroptosis-LN” intersection genes

The 19 “ferroptosis-LN” intersection genes acquired from Section 3.4 were entered into the UniProt database, transformed into complete protein names, and subsequently subjected to a search for TCMs within the TCMSp database. Among these, 9 genes failed to yield relevant TCM matches, and duplicate TCM entries were omitted. Ultimately, a compilation of 412 TCMs aligned with 10 “ferroptosis-LN” intersection genes was amassed. For detailed information, please refer to Figure 5. Data analysis revealed that the top 5 TCMs containing the highest gene count were Mahuang, Gehua, Baiguo, Chuannxiuxi, and Jinyinhua, as illustrated in Figure 6.

“TCM-target” network and “TCM-LN” PPI network construction

Utilizing the TCMSp database, Mahuang, Gehua, Baiguo, Chuannxiuxi, and Jinyinhua yielded 69 active ingredients and 334 targets collectively. This interaction is depicted in the “TCM-target” network, exemplified in Figure 7. Employing VENNY 2.1.0 software, a total of 43 overlapping genes from the “TCM-LN” intersection were identified and subsequently entered into the STRING database for the establishment of the “TCM-LN” PPI network, showcased in Figure 8. Following the degree value assessment, the leading 15 genes were identified as the core genes within the “TCM-LN” context. For an in-depth overview, consult Table 2.

Acquisition of key genes for the regulation of ferroptosis associated with LN by TCM

The 19 “ferroptosis-LN” intersection genes acquired in Section 3.4 and the leading 15 “TCM-LN” core genes obtained from Section 3.7 were entered into the VENNY 2.1.0 software, specifically IL1β, TLR4, IFNG, STAT3, and HMOX1, as displayed in Figure 9.

Discussion

In TCM, LN falls under the categories of “yin yang du”, “shui zhong”, and “hu die ban” [12]. Research indicates that 60–80% of SLE patients may encounter renal impairment, with around 20% of LN patients progressing to end-stage renal disease [13]. Ferroptosis, a novel pathogenic mechanism, has been identified as a contributor to the development of various ailments. It has shown associations with several autoimmune disorders, including SLE, rheumatoid arthritis, and inflammatory bowel disease [7, 14–16].

In this investigation, we employed multi-database mining and literature exploration to dissect the “ferroptosis-LN” intersection genes. The PPI network diagram unveiled pivotal potential genes within the “ferroptosis-LN” context, such as IL-6, IL-1β, TLR4, STAT3, and more. The absence of immune tolerance in T and B cells among LN patients triggers the activation of diverse cytokines (e.g., IL-6, IL-1β, IL-10) by various helper T cells, exacerbating inflammation progression within renal tissues [17]. Previous studies have pointed out significantly elevated serum levels of inflammatory factors like IL-6 in LN patients [18]. The deficiency of immune tolerance to self-antigens in LN patients prompts the binding of Toll-like Receptors to their ligands, initiating intracellular signalling, and subsequently triggering the NF-κB signalling pathway. This cascade results in extensive proliferation of immune cells and the release of various cytokines, thereby instigating inflammatory damage within renal tissue [19]. TLR4 has also been implicated in activating renal parenchymal cells and intensifying the deposition of immune complexes in the kidney [20].

The JAK2/STAT3 signalling pathway participates in various inflammatory responses and immune regulatory processes [21]. GO and KEGG enrichment analyses have revealed that ferroptosis genes predominantly participate in inflammatory responses, cellular activation, positive modulation of chemokine production, and negative regulation of immune system processes within LN's pathology. This linkage could be tied to the accumulation of ferroptosis lipid ROS and GPX4 processes. Lipid peroxidation, driven by lipid ROS, has been shown to induce an inflammatory response by facilitating the release of HMGB1 [22]. In addition, animal investigations have uncovered that GPX4 deficiency leads to a marked elevation in levels of the inflammatory factor IL6 and monocyte chemotactic protein-1, thus inducing an inflammatory response [23]. These explorations suggest that ferroptosis exerts a pro-inflammatory influence.

KEGG pathway enrichment analysis has unveiled the principal pathways contained in LN, including Necroptosis, Inflammatory bowel disease, Pathways in cancer, HIF-1 signalling pathway, and the Ferroptosis pathway. Necroptosis, a form of programmed cell death, is impacted by a range of factors like ROS, inflammatory factors, and tumour necrosis factor-alpha [24]. Both necroptosis and ferroptosis can contribute to the body’s immune response. Activation of the necroptosis pathway can lead to considerable inflammation, while ferroptotic cells release inflammatory mediators via esterase and cyclooxygenase-mediated arachidonic acid metabolism, thereby prompting immune responses [25, 26]. Research has indicated that gut microbiota holds associations with diverse immune disorders. Animal studies have uncovered correlations between LN severity and reductions in lactic acid bacteria and Lachnospira populations in the body [27]. Disruptions in the intestinal flora of LN patients might lead to diminished body immunity to the microbiota and trigger a systemic inflammatory reaction [28]. HIF-1 plays a pivotal role in cellular adaptation to oxygen supply and can influence gene expression [29]. Investigations have also established ferroptosis’s potential to eliminate cancer cells, thus offering a strategy to manipulate cellular ferroptosis for cancer treatment [30, 31].
Figure 7 Network of “TCM-target”. A1: Active ingredient targets common to MH, GH, BG, CNX, JYH; B1, B2: Active ingredient targets common to MH, GH, JYH; C1: Active ingredient targets common to MH, JYH; D1: Active ingredient targets common to MH, GH, CNX, JYH; E1: Active ingredient targets common to BG, JYH. TCM, traditional Chinese medicine; MH, mahuang; GH, gehua; BG, baiguo; CNX, Chuanniuxi; JYH, jinyinhua.

Figure 8 The PPI network of commonly related genes in “TCM-LN”. The larger the genes area, the darker the corresponding colour, representing the greater the number of associations of the gene with other genes. The wider the width of the line, the darker the corresponding colour, representing the greater the degree of continuity between the two genes. TCM, Traditional Chinese medicine; LN, lupus nephritis.

Figure 9 Venn diagram showing commonly related genes in “TCM-LN” and “Ferroptosis-LN”. TCM, traditional Chinese medicine; LN, lupus nephritis.
Table 2 Commonly related genes information of “TCM-LN”

<table>
<thead>
<tr>
<th>Number</th>
<th>Gene Name</th>
<th>Full name of the gene</th>
<th>Degree</th>
<th>Node Tightness</th>
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<td>Albumin</td>
<td>39</td>
<td>0.953</td>
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<td>IL-6</td>
<td>Interleukin 6</td>
<td>38</td>
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<td>Tumor Necrosis Factor</td>
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<td>0.932</td>
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<td>IL1β</td>
<td>Interleukin 1β</td>
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<td>0.891</td>
</tr>
<tr>
<td>5</td>
<td>TLR4</td>
<td>Toll-like receptor 4</td>
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<td>0.891</td>
</tr>
<tr>
<td>6</td>
<td>AKT1</td>
<td>RAC-alpha serine/threonine protein kinase</td>
<td>36</td>
<td>0.891</td>
</tr>
<tr>
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<td>VEGFA</td>
<td>Vascular endothelial growth factor A</td>
<td>35</td>
<td>0.872</td>
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<td>Vascular cell adhesion protein 1</td>
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<td>HMOX1</td>
<td>Heme oxygenase 1</td>
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</table>

TCM, traditional Chinese medicine; LN, lupus nephritis.

The TCMP database was employed to procure the five most closely associated TCMs for the pivotal gene within the “ferroptosis-LN” nexus, specifically Mahuang, Gehua, Baiguo, Chuannixi, and Jinyinhu, acknowledged for their anti-inflammatory and anti-cancer properties [32–35]. VENN software was employed to discern five common genes shared between “ferroptosis-LN” and “TCM-LN”, which encompassed IL1β, TLR4, IFNG, STAT3, and HMOX1. It has been established that IL1β, TLR4, IFNG, and STAT3, functioning as inflammatory factors, participate in an inflammatory response leading to the production of inflammatory mediators [36]. HMOX1 contributes to the transformation of haemoglobin into free ferrous iron within the oxidative stress response, subsequently intensifying the oxidative stress reaction [37]. Moreover, LncRNA-2870/miR-3587/HMOX1 has been identified to regulate renal ischemia-reperfusion-induced ferroptosis [38].

Nevertheless, certain limitations are associated with our study. Initially, we did not comprehensively delineate the TCM-mediated ferroptosis pathway in LN. Moreover, the identification of ferroptosis-related genes relied solely on the FerrDb database, while LN-related genes were extracted from OMM, Gene Cards, and Drug Bank. The exclusion of numerous databases marks another limitation. Lastly, the study was devoid of validation experiments.

Conclusion

In this study, the shared genes within the context of “TCMs-LN-ferroptosis” were determined using network pharmacology analysis and data mining techniques. Among these, Mahuang, Gehua, Baiguo, Chuannixi, and Jinyinhu might impact the progression of LN by modulating cellular ferroptosis processes. Furthermore, TCMs could potentially affect LN’s progression by intervening in the regulation of key ferroptosis genes such as IL1β, TLR4, IFNG, STAT3, and HMOX1. However, further research is imperative to validate these findings conclusively.

References

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