Tropical Journal of Pharmaceutical Research February 2024; 23 (2): 327-337
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i2.12

Original Research Article

Exploring the mechanism of action of *Tripterygium wilfordii* Hook F in the treatment of rheumatoid arthritis based on network pharmacology and molecular docking

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Sent for review: 18 September 2023 *Revised accepted: 2 February 2024*

Abstract

Purpose: *To determine the mechanism of action of Tripterygium wilfordii Hook F (TwHF) in the treatment of rheumatoid arthritis (RA) based on network pharmacology and molecular docking.*
Methods: *The active constituents and targets of TwHF were screened by searching the TCMSP, TCMIP, PharmMapper database, and BATMAN-TCM platform combined with oral bioavailability and drug-like analysis. The drug-component-target maps were drawn using the UniProt database and Cytoscape 3.9.0 software. The drug-target maps were searched in GeneCards, OMIM, TTD, PharmGKB, and DrugBank databases to obtain the predicted targets of RA, Venn diagrams were drawn to derive the common targets of TwHF components and RA and protein-protein interaction (PPI) network, GO enrichment as well as KEGG pathway analyses were performed. The potential binding activities between the active constituents of TwHF and the targets were predicted using molecular docking.*
Results: *Seven active components and 131 potential targets were found for TwHF while RA had 4,917 related targets. However, TwHF and RA had 87 common targets. The target genes obtained from the PPI network include tumor necrosis factor (TNF), p53 tumor protein (TP53) and vascular endothelial growth factor A (VEGFA). The GO enrichment and KEGG pathway analysis yielded 336 results and 121 signal pathways, respectively.*
Conclusion: *Tripterygium wilfordii Hook F therapy for RA may be a multi-component, multi-target and multi-signal pathway biological process, which may regulate VEGFA, TNF, TP53 and other targets and also exhibit anti-inflammatory and immunomodulatory functions amongst others. Future studies should determine the relationship of the identified targets in vivo to produce alternative treatments for RA.*

Keywords: *Rheumatoid arthritis, Network pharmacology, Molecular docking, Bioavailability*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovitis and the destruction of cartilage [1]. Currently, the treatment of RA is mainly aimed at alleviating clinical symptoms and improving disease progression. Western medical treatment drugs

for RA mainly include disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs, glucocorticoids and corticosteroids [2]. These drugs are expensive and their long-term use exerts adverse effects, so it is necessary to explore new treatment modalities. As the treatment of RA by traditional Chinese medicines is gradually gaining attention and has the advantage of high efficiency and low cost, it is generally favored by patients with RA. *Tripterygium wilfordii* Hook F (TwHF) refers to a fat-soluble mixture (containing Triptolide) extracted from this plant of the Family Eytidae. It is the first Chinese herbal medicine with anti-inflammatory, immunomodulatory and bone protection effects studied in China [3]. Recent studies have reported the efficacy of TwHF in the treatment of immune-related diseases, such as chronic urticaria and rheumatoid arthritis [4]. The main active ingredient of TwHF is diterpene lactone and it has been widely used in clinical practice, especially as a common treatment for RA due to its anti-inflammatory and immunomodulatory properties. Although its efficacy is well established, its specific mechanism of action is unclear. This study aimed to explore the key targets and mechanism of action of TwHF in treating RA and to provide a reference for further basic experimental research and rational clinical application of TwHF.

METHODS

Screening of active components and targets of TwHF

This screening was done through the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php), traditional Chinese medicine database platform (TCMIP, http://www.tcmip.cnl) and PharmMapper database (http://lilab.ecust.edu.cnlpharmmapperlindex.php) [5]. First, a database search was performed for "Lei Gong Deng " to obtain all chemical components of TwHF. Oral bioavailability (OB) set at ≥ 30% and drug-likeness (DL) at ≥ 0.18 were used to screen the active constituents of TwHF and query the databases for the main active constituents in order to obtain the relevant targets. Finally, the target proteins were transformed into standardized gene names using the UniProt database (https://www.uniprot.org).

Screening of RA-related targets

In the OMIM (https://www.omim.org), GeneCards (https://www.genecards.org), TTD (https://db.idrblab.net/ttd), PharmGKB (https://www.pharmgkb.org) and DrugBank (https://go.drugbank.com) databases, a comprehensive search was done using the theme word "rheumatoid arthritis" by selecting the species as "human origin" and "gene" option, to retrieve all the target information related to RA. Finally, EXCEL software was used to sort out and remove duplications. The results from five different databases were summarized to obtain the final target.

Screening of "drug-disease" intersection targets

The screened TwHF active constituents and RA target genes were mapped through the Ven 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) website to obtain the intersecting target genes. Then, the "Chinese Medicine Compounds-Action Targets" network was constructed using the Cytoscape 3.9.0 software.

Construction of a network diagram of PPIs

The STRING online database (https://cn.string-db.org/) was used to build a PPI network diagram. First, the multiple Proteins column and TwHF and RA intersection targets obtained were inputted into the project box. Next, in the “organisms” category, "Homo sapiens" was selected with other parameters left at the default setting. The drugs obtained and the disease PPI network data were exported in TSV format, and the Cytoscape 3.9.0 software was used to plot the mesh and analyze key targets based on degree values.

GO and KEGG enrichment analyses

On the DAVID online data platform (https://david.ncifcrf.gov/), the "Shortcut to DAVID Tools" was selected from the project bar. Thereafter, the intersection targets of TwHF and RA were imported into the database and the threshold was set to $p < 0.05$. To proceed, the "Select Identifier" column (official gene symbol) was checked while "Homo sapiens" was selected in the "Select species" column. Then, functional enrichment of Genes Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed.

Molecular docking

To identify the ligand, the TCMSP database was used to export the 2D structure of the TwHF core target. Then, the 2D structure was downloaded from the PubChem web page (https://pubchem.ncbi.nlm.nih.gov/) in "SDF" format and Chem3D software was used to

convert the 2D structure into a 3D structure. In order to determine the receptor, the core target was imported into the UniProt database. Thereafter, the protein molecular data was downloaded in the "PDB" format through the PDB database (https://www.rcsb.org/) while PyMOL software was used to "dehydrate" and "remove impurities" of the PDB protein molecules. Finally, the collected ligands and receptors were introduced into AutoDock Tools software for processing and the 3D binding diagram was drawn using PyMOL software.

RESULTS

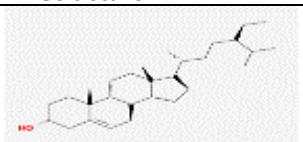
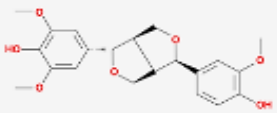
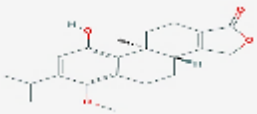
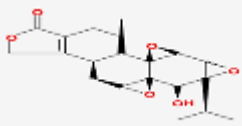
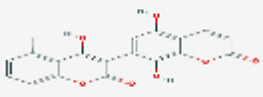
Screening of active constituents and targets of TwHF

A total of 44 active constituents were obtained by searching the TCMSP, TCMIP and PharmMapper databases. Based on the active ingredient screening conditions of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18, seven TwHF active constituents were screened out (Table 1). Results of the selected active constituents were compiled and target gene matching was performed using UniProt data platform to remove duplicate target proteins and non-human target proteins. Finally, 155 potential targets were obtained.

RA disease target screening

A total of 41 targets (OMIM), 4882 targets (GeneCards), 177 targets (TTD), 9 targets (PharmGKB) and 595 RA targets (DrugBank) were. A total of 4,917 RA targets were obtained after merging and removing duplicates. The selected TwHF targets and RA targets were mapped using the Venn 2.1.0 website and then the Venn diagram was drawn (Figure 1). A total of 87 intersection targets were obtained from the Venn diagram out of which the top 15 intersection targets are listed in Table 2.

Table 1: Active constituents of TwHF

Mol ID	Structure	Active Ingredients	OB%	DL
MOL000296		Progesterone receptor	36.91	0.75
MOL003182		Coagulation factor Xa	60.69	0.62
MOL003184		Delta-type opioid receptor	45.42	0.53
MOL003185		Interleukin-8	48.84	0.38
MOL003187		Monocyte differentiation antigen CD14	51.29	0.68
MOL003196		Interstitial collagenase	48.5	0.44
MOL003199		Peroxidase C1A	61.85	0.54

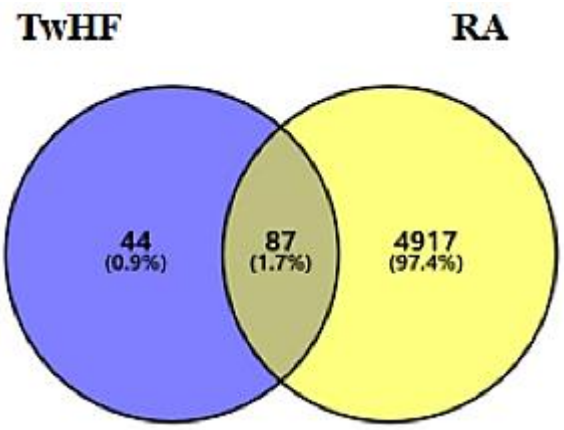


Figure 1: Venn diagram of active constituents of TwHF and RA intersection targets

"Drug component-target gene-disease" network diagram

The 87 intersecting targets from the Venn diagram were used to create a "drug component-target gene-disease" network using the Cytoscape 3.9.0 software (Figure 2). Based on this network topology, the top 10 genes were plotted in a histogram using the degree value as a reference standard (Figure 3). The compounds with higher degree values were microRNA 132 (MIR-132), peptidyl arginine deaminase-4 (PAD-l4) and microRNA 155 (MIR-155), indicating that TwHF and RA act synergistically via these components.

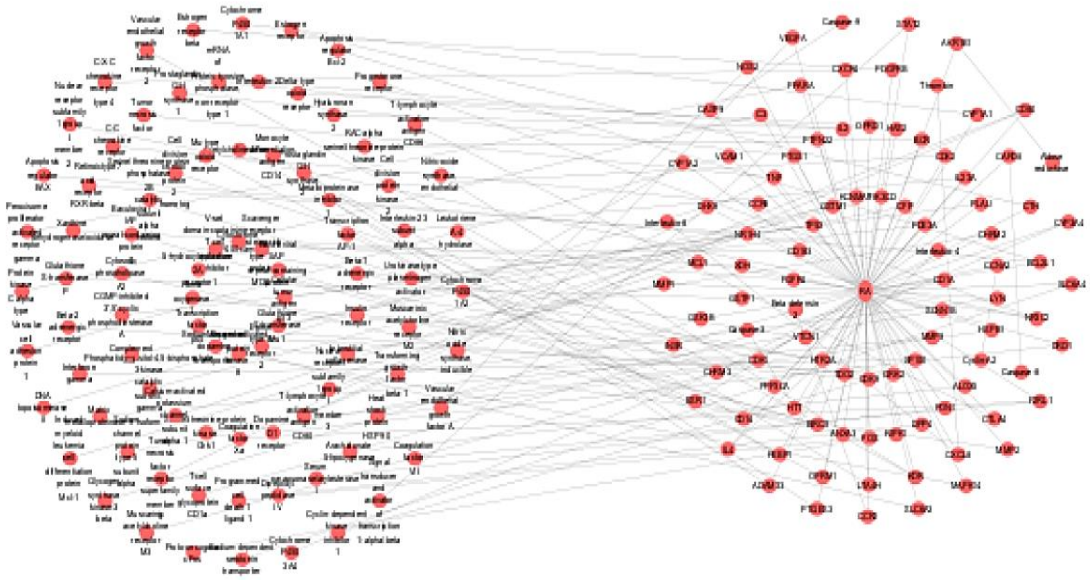


Figure 2: "Active ingredient-target-RA" network

Table 2: Intersection of the active ingredient of TwHF and RA genes

Number	Protein	Genes	Uniprot ID
1	Progesterone receptor	NR3C2	P08235
2	Muscarinic acetylcholine receptor M3	CHRM3	P20309
3	Gamma-aminobutyric-acid receptor alpha-3 subunit	CHRM2	P08172
4	Alcohol dehydrogenase 1C	PTGS1	P23219
5	Lysozyme	SCNN1B	P51168
6	Nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase	PTGES3	Q15185
7	Sodium channel protein type 5 subunit alpha	PDE3A	Q14432
8	Retinoic acid receptor RXR-alpha	FGFR4	P22455
9	Sodium-dependent noradrenaline transporter	F2RL1	P55085
10	Cytochrome P450-cam	KDR	P35968
11	Potassium voltage-gated channel subfamily H member 2	DHX9	Q08211
12	Coagulation factor Xa	HSPB1	P04792
13	Calmodulin	OPRD1	P41143
14	Muscarinic acetylcholine receptor M5	GRK2	P25098
15	Carbonic anhydrase II	OPRM1	P35372

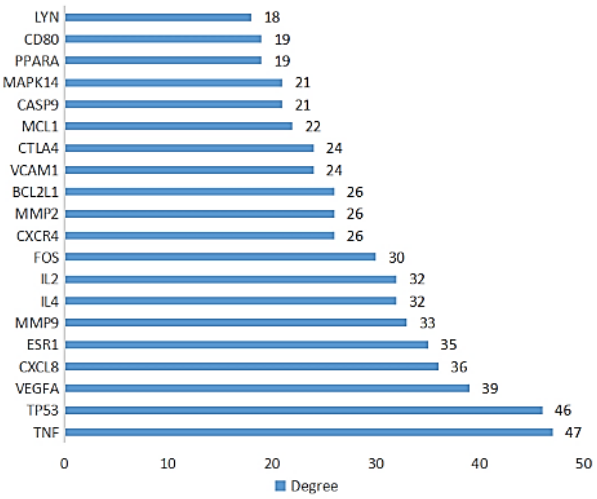


Figure 3: Histogram of the top 20 genes

PPI network diagram construction

The active components of TwHF and the core targets of RA were used to construct PPI network maps and key protein maps in PPI were analyzed using the String online platform and Cytoscape 3.9.0 software (Figure 4 and Figure 5). The top three targets, in terms of degree, were tumor necrosis factor (TNF), p53 tumor protein (TP53) and vascular endothelial growth factor-A (VEGFA).

GO function and KEGG enrichment analyses

The 87 intersection targets were imported into the DAVID database for GO function and KEGG enrichment analyses. Gene Ontology (GO) function is mainly divided into three aspects: cellular component (CC), molecular function (MF) and biological process (BP) of which BP returned kidney development and positive regulation of calcineurin-NFAT signaling cascade and endocytosis. The MF showed G protein β-subunit binding, transcription regulatory region sequence-specific DNA binding and kinase activity while CC was composed of the nucleus, the perinuclear region of the cytoplasm and the early endosome (Figure 6). The KEGG pathway enrichment analysis revealed the enrichment of the nuclear factor kappa-B (NF-κB), Toll-like receptor (TLR) and vascular endothelial growth factor (VEGF) signaling pathways (Figure 7).

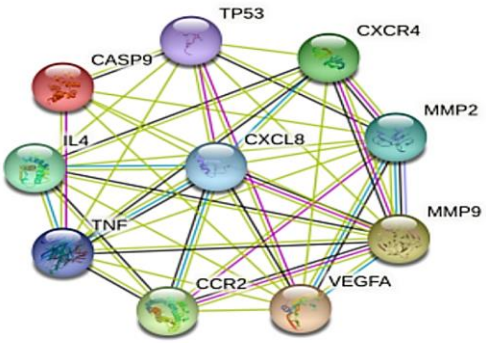


Figure 5: Key proteins in PPI

Molecular docking

The top three active constituents from the "active ingredient-target" network diagram (micro-ribonucleic acid 132, peptide arginine deimidase 14 and micro-ribonucleic acid 155) were connected to the key targets TNF, TP53 and VEGFA obtained via topology analysis.

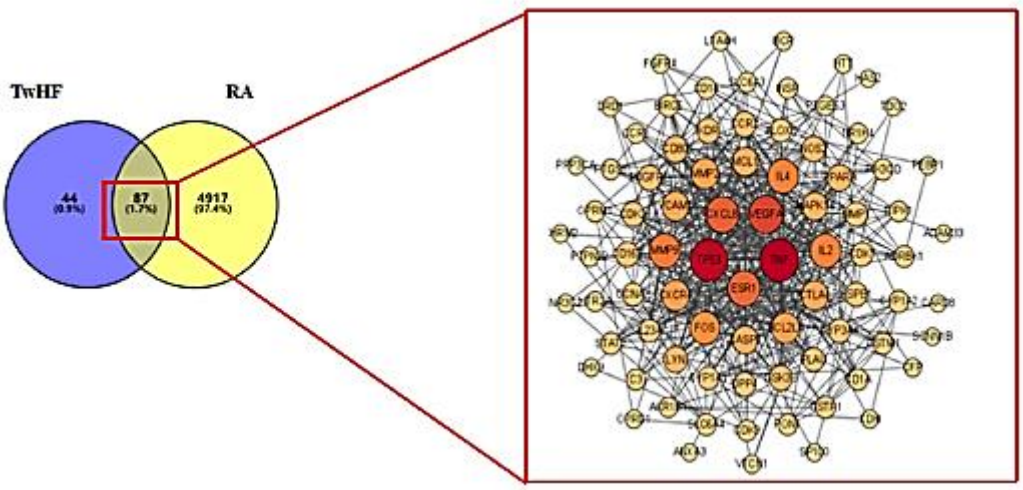


Figure 4: PPI network diagram

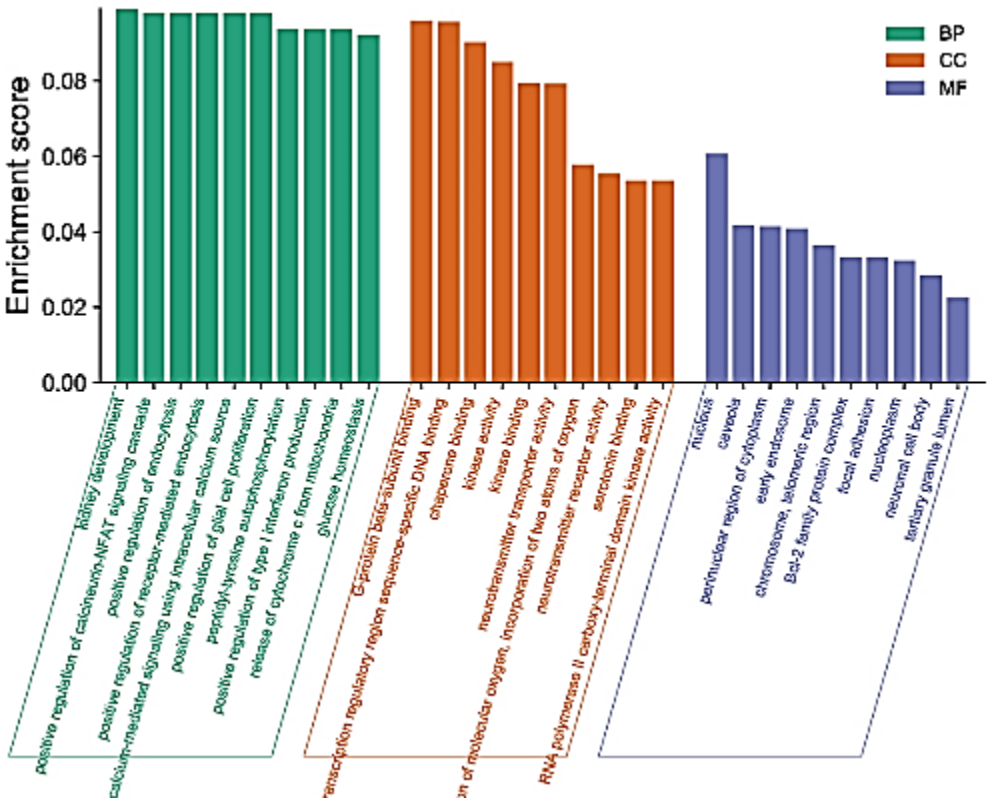


Figure 6: GO function enrichment analysis

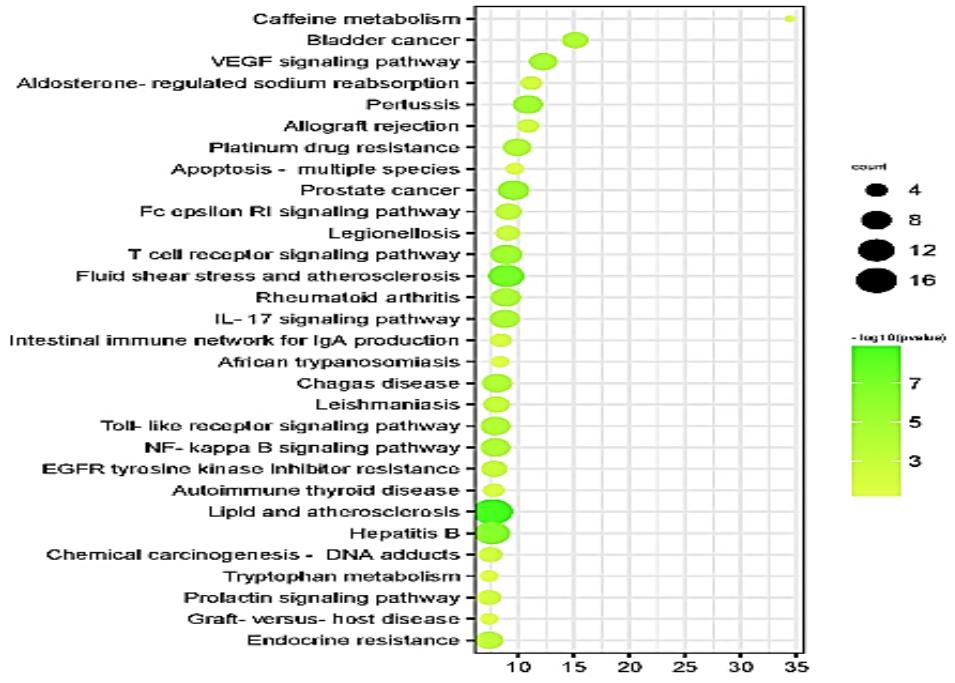


Figure 7: KEGG pathway enrichment analysis

The resulting output was optimized using PyMOL software. The lower the minimum binding energy, the higher the binding activity between the target protein and the active ingredient, indicating that the binding capacity between the two is better [6]. The results showed the possibility of hydrogen bonding, π-π conjugate, hydrophobic accumulation and other intermolecular binding forces between the active ingredient and the target, indicating that the target protein has strong binding energy with the main active ingredient (Figure 8).

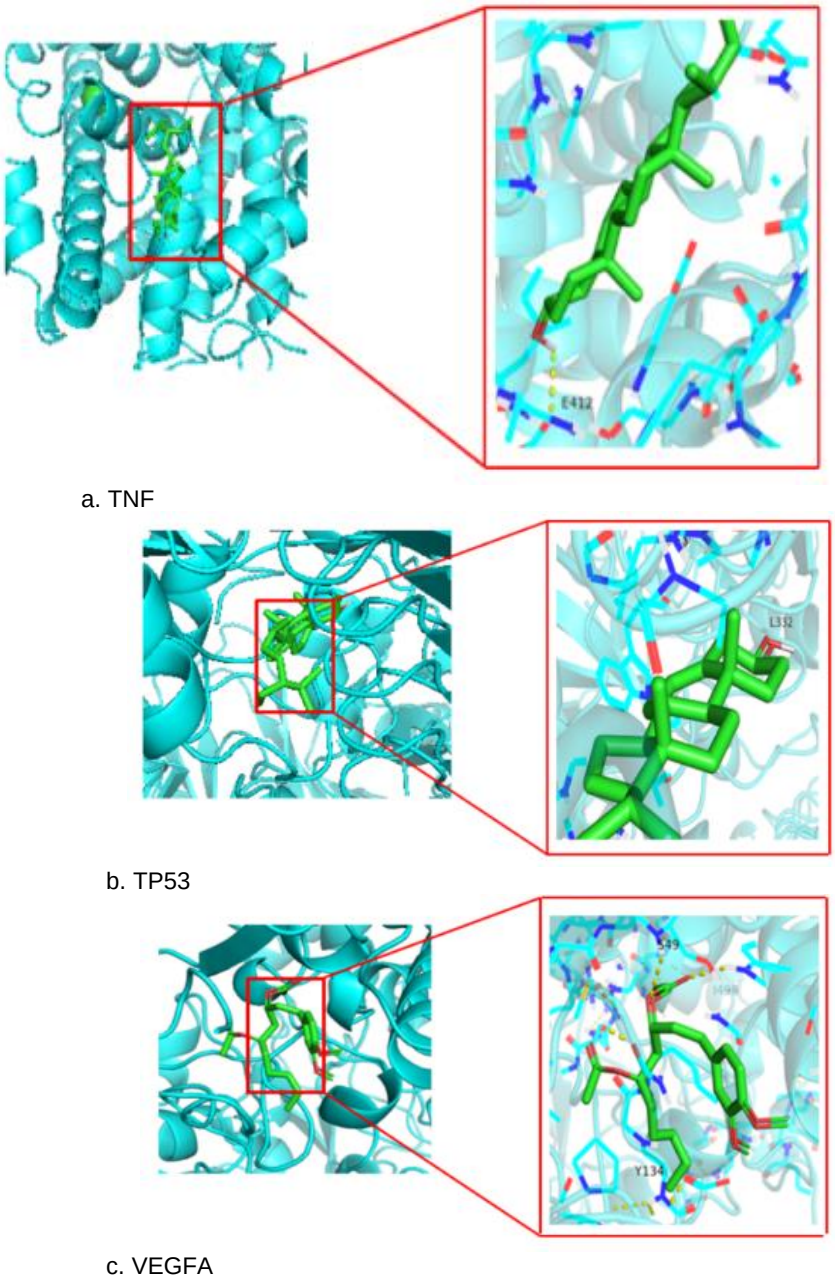


Figure 8: Molecular docking of active constituents of TwHF and key targets

DISCUSSION

Early treatment and attainment therapy are currently the main treatment strategies for RA, with complete remission or low disease activity as the treatment goal. The current treatment for RA is mainly based on modern drug therapies, but they require prolonged exposure and disease may be resistant to some of these drugs [7]. Hence, the urgent need to search for new anti-RA drugs remains to be addressed by researchers. It is difficult to achieve good clinical efficacy with single-target drugs, so multi-component, multi-target drugs may become a trend in the treatment of RA. *Tripterygium wilfordii* Hook F (TwHF) was first referred to in the book *Dian Nan Ben Cao*, which was written by Lan Mao in the Ming Dynasty. It is described as a plant with pungent taste, warm in nature and poisonous. It enters and travels the twelve meridians of the liver and spleen. So far, more than 100 kinds of components, such as alkaloids, diterpenoids, triterpenoids and polysaccharides, have been isolated from the plant *Ligustrum*. In recent years, studies have confirmed the immunosuppressive, anti-inflammatory, antioxidant, analgesic and neuroprotective effects of various components of TwHF [8], but the potential mechanisms, biological processes and metabolic pathways by which the active

components of TwHF improve the physicochemical indices of RA are still not clear.

Network pharmacology provides a new strategy to study Chinese medicine from a multi-target approach [9]. In this study, the "drug component-target-disease" network diagram revealed that the active components of TwHF involved in treating RA were mainly MIR-132, PAD-l4 and MIR-155. Amongst them, miRNA is an endogenous non-coding small RNA that is involved in disease pathogenesis by regulating the transcription and translation of mRNA, regulates various stages of immune cell maturation and maintenance of immune homeostasis, and is closely related to the development of autoimmune diseases [10]. In addition, miRNAs can play an important role in the pathophysiology of patients with RA by regulating matrix metalloproteinases (MMPs). Chatzikyriakidou and co-workers found that miR-132 plays an important role in the pathophysiology of peripheral blood mononuclear cells (PBCs) in patients with RA [11]. The expression of miR-132 was found to be upregulated in the peripheral blood mononuclear cells (PBMC) of patients with RA and the polymorphism of its target gene was closely related to the pathogenesis of RA, suggesting that miR-132 could be a molecular marker of RA disease activity [12]. Elmesmari *et al* [13] showed that miR-155 was highly expressed in peripheral blood and monocytes of patients with RA and could promote the accumulation of inflammatory cells in the synovial membrane through the regulation of chemokine and pro-inflammatory chemokine receptor expression, triggering the disease.

Studies have shown that upregulation of serum exosome miR-155 expression may be involved in the development of RA disease, providing an insight into the possibilities of how serum exosomes can regulate the occurrence and development of RA disease [14]. As a member of the PAD family, PAD-l4 is an important modified enzyme after protein translation, which is mainly distributed in the cytoplasm of macrophages, granulocytes and monocytes, and catalyzes the conversion of arginine into citrulline residues. There is a close relationship between disorders of PAD-l4 activity and the development of RA disease, and studies have shown that the susceptibility gene of RA is associated with PAD-l4, which is expressed in blood and RA synovial tissue. According to the analysis of the PPI network diagram, the top three targets based on degree values were TNF, TP53 and VEGFA. Vascular Endothelial Growth Factor (VEGF) is a highly specific pro-vascular endothelial cell growth factor that promotes increased vascular permeability, extracellular matrix degeneration, vascular endothelial cell migration and proliferation, and angiogenesis. The VEGFA is a member of the VEGF family that selectively acts on vascular endothelial cells and stimulates angiogenesis *in vitro* and *in vivo*, playing an active role in the induction, maintenance and growth of vascular endothelial cells [15].

Studies have shown that VEGFA cannot only promote the formation of RA synovial vascular fender but also acts as one of the direct pro-inflammatory factors for the progression of RA disease, which can protect synovial vascular fibrous cells from apoptosis and promote synovial cell proliferation [16]. It interacts with the inflammatory cytokines of RA and directly or indirectly regulates the expression of VEGF, thereby promoting the production of RA blood vessels. Tumor necrosis factor (TNF) is one of the factors that induce apoptosis, which is involved in a variety of pathogenic mechanisms in the development of RA disease. It can activate the synovial cells, macrophages, chondrocytes and osteoclasts in RA joint tissue, leading to local inflammatory responses and vascular formation, causing cartilage destruction and bone erosion. At present, biologics represented by TNF inhibitors can effectively improve the condition of RA and many clinical trials have confirmed that TNF inhibitors can improve joint inflammation and joint function, reduce the clinical activity of RA and delay the radiological progression of joints.

The *TP53* gene is an oncosuppressor and it encodes the p53 protein. Its main role is to regulate the cell cycle, repair damaged DNA, induce apoptosis and inhibit angiogenesis. Studies have shown that the *TP53* gene affects apoptosis of RA synovial fibroblasts in rat models of adjuvant arthritis (AA) and may alleviate disease progression if the *TP53* gene is overexpressed at early stages in the disease [7]. Based on the above results, a synergistic effect between the active components of TwHF was observed, which may be effective in treating RA by reducing the levels of inflammatory transmitters and immune regulation.

Gene Ontology (GO) functional enrichment analysis revealed that the BPs targeted by TwHF in the treatment of RA mainly involved kidney development, positive regulation of calcineurin-NFAT signal cascade and positive regulation of endophagy. On the other hand, MF mainly involved G protein β subunit binding, transcriptional regulatory region sequence-specific DNA binding and kinase activity. The

KEGG enrichment analysis revealed that the signal pathways involved are the NF-κB, TLR, and VEGF signal pathways. Nuclear factor kappa B (NF-κB) is an important downstream protein of multiple signaling pathways involved in the transcription of cytokines, adhesion molecules and proteases that regulate various inflammatory responses. It is highly activated in the RA synovium and induces the secretion of a variety of pro-inflammatory factors, resulting in synovitis and joint destruction [17].

Tripterygium wilfordii Hook F (TwHF) modulates the activation of the NF-κB signaling pathway and the production of inflammatory factors such as IL-1β and TNF-α in RA by inhibiting the expression of nicotinic acetylcholine receptors α7 (nAChRα7) in patients with RA. The TLR signaling pathway plays an important role in the progression of RA and the absence of a death domain of myeloid differentiation factor 88 (MyD88) in the TLR signaling pathway can lead to limited recruitment activity of downstream interleukin (IL)-1 receptor-associated kinases, weakening the action of IL-6 IL-12, and TNF-α. In addition, TwHF extracts have been found to reduce the production of inflammatory transmitters and inflammation by inhibiting signaling factor expression in the TLR4/NF-κB signaling pathway. The VEGF signaling pathway is one of the most powerful positive regulatory pathways, playing an irreplaceable role in inducing angiogenesis, promoting the proliferation and migration of vascular endothelial cells and improving vascular permeability [18].

In summary, network pharmacology and molecular docking methods were applied to conduct exploratory studies on the active components, targets, and signaling pathways of TwHF in the treatment of RA. Various active components in TwHF were found to positively regulate endophagy, G protein β subunit binding, transcriptional regulatory region sequence-specific DNA binding and kinase activity, and comprehensively targeted regulation through the NF-κB, TLR and VEGF signaling pathways. These components, therefore, have a potential therapeutic role. This study preliminarily explores the mechanism of action of TwHF in the treatment of RA and also provides new ideas and references for the in-depth study of TwHF.

CONCLUSION

In silico analysis shows that *Tripterygium wilfordii* Hook F positively regulate endophagy, G protein β subunit binding, transcriptional regulatory region sequence-specific therapy through the NF-κB, TLR and VEGF signaling pathways. This study is a preliminary work that employed *in silico* techniques to identify the components, targets and signaling pathways of TwHF in the treatment of RA. Future studies should determine the relationship of the identified targets *in vivo* with a view to producing alternative treatment for RA.

DECLARATIONS

Acknowledgements

This work was supported by the National Natural Science Foundation of China (nos. 82160869 and 82374494; Guizhou Provincial Key Technology R&D Program, Qiankehe Support (2021, no. general 006; Guizhou Provincial Basic Research Program(Natural Science) Qiankehe Basics-ZK (2023, no. general 412; Guikehe Academic New Miao (no. 2023-36); National and Provincial Scientific and Technological Innovation Talent Team Training Project of Guizhou University of Traditional Chinese Medicine, Guizhou Traditional Chinese Medicine TD Hezi (2022, no. 004).

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets generated during and/or analyzed in the current study are available in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php), traditional Chinese medicine database platform (TCMIP, http://www.tcmip.cnl), PharmMapper database (http://lilab.ecust.edu.cnlpharmmapperlindex.php), OOMI (https://www.omim.org), GeneCards (https://www.genecards.org), TTD (https://db.idrblab.net/ttd), PharmGKB (https://www.pharmgkb.org), and DrugBank databases (https://go.drugbank.com) repository.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors. The conceptualization process: Wu-kai Ma, and Xue-ming Yao; methodology: Xue-mei Yuan, Cong Huang; data analyses: Xue-mei Yuan, Chang-ming Chen; validation: Wu-kai Ma, Xue-ming Yao, and Feng Luo; formal analysis: Hong Xiong; investigation: Xue-mei Yuan; data curation; Feng Luo; writing: first draft preparation, Feng Luo; writing—review and editing; Wu-kai Ma and Xue-ming Yao. This manuscript was reviewed and approved by all authors for publication.

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