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Exploring the mechanism of action of TwHF in the treatment of rheumatoid arthritis based on network pharmacology and molecular docking

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Abstract

objectives: To explore the Relationship and Potential Mechanism of Tripterygium Wilfordii Hook F (TwHF) in the Treatment of Rheumatoid Arthritis (RA) Based on Network Pharmacology and Molecular Docking.

Methods: The main active ingredients and targets in TwHF were screened by searching the TCMSP database, combined with oral bioavailability and drug-like analysis, and then the drug-component-target maps were drawn by UniProt database and Cytoscape 3.9.0 software; the drug-target maps were searched in GeneCards, OMIM, TTD, pharmGKB, and drugbank databases. drugbank database to obtain the predicted targets of RA, draw Venn diagrams to derive the common targets of TwHF components and RA, and perform protein-protein interaction (PPI) network analysis, GO enrichment and KEGG pathway The potential binding activities between the active ingredients of TwHF and the targets were predicted by molecular docking technique.

Results: TwHF had 7 active ingredients and 131 potential targets, RA had 4917 related targets, and TwHF and RA had 87 common targets. The target genes obtained by the PPI network include tumor necrosis factor (TNF), p53 tumor protein (TP53), vascular endothelial growth factor A (VEGFA), etc.; GO enrichment and KEGG pathway analysis yielded 336 results and 121 signal pathways, respectively.

Conclusion: TwHF therapy for RA may be a multi-component, multi-target, multi-signal pathway biological process, it may be able to regulate VEGFA, TNF, TP53 and other targets, play anti-inflammatory, immunomodulatory and other functions.

Background

RA is a chronic autoimmune disease characterized by synovitis and cartilage destruction[1].Currently, the treatment of RA is mainly aimed at alleviating clinical symptoms and improving disease progression, and western medical treatment drugs are mainly disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs and glycocorticosteroids,Corticosteroids, etc[2],but the long-term use of such drugs is not only adverse effects, but also expensive,so it is practical to explore new treatment modalities. As the treatment of RA by traditional medicine is gradually gaining attention and has the advantage of high efficiency and low cost, it is gradually favored by RA patients. TwHF is the root of Radix Rehmanniae, family Weimaraceae, which has the effects of dispelling wind and removing dampness, activating blood circulation, reducing swelling and pain, etc. It has been clinically applied in the treatment of RA forits various effects such as anti-inflammation and immunomodulation, and has achieved good results, but its specific mechanism of action is not clear[3-5]. In this study, we used TCMSP, GeneCards, OMIM, TTD, pharmGKB, drugbank andother databases to mine TwHF and RA-related genes, used Cytoscape to construct specific target networks of TwHF for RA treatment, constructed protein-protein interaction (PPI) network maps of both, and performed GO function enrichment analysis The aim

is to explore the key targets and mechanism of action of TwHF in the treatment of RA, and to provide reference for further basic experimental research and rational clinical application of TwHF.

1 Information And Methods

1.1 Schematic diagram of method

The schematic diagram of research methods is shown in Fig. 1.This study explored the mechanism of action of Tripterygium wilfordii in the treatment of rheumatoid arthritis by using network pharmacological methods and molecular docking.

1.2 Screening of active ingredients and targets of TwHF

Through the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://old.tcmsp-e.com/tcmsp.php) [6],we searched for "Lei Gong Deng ", obtain all chemical components of TwHF, and set oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 to screen the active ingredients of TwHF drugs, and query the main active ingredients in this database for relevant targets; finally, the obtained target proteins were transformed into standardized gene names using the UniProt database (https://www.uniprot.org).

1.3 Screening of RA-related targets

In the OOMI database (https://www.omim.org), GeneCards database (https://www.genecards.org), TTD database (https://db.idrblab.net/ttd), pharmGKB database (https://www.pharmgkb.org), In the drugbank database (https://go.drugbank.com), set the theme word "rheumatoid arthritis", by selecting the species as "human origin" and "gene", deriving all the target information related to RA, and finally using EXCEL to sort out and deduplication, summarize the 5 databases to obtain the final target.

1.4 Screening of "drug-disease" intersection targets

The screened TwHF active ingredient 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, and then the "Chinese Medicine Compounds-Action Targets" network was constructed using Cytoscape 3.9.0 software.

1.5 Construction of a network diagram of protein-protein interaction (PPI).

Through the STRING online database (https://cn.string-db.org/) to build a PPI networkdiagram, first select the multiple Proteins column, the obtained TwHF and RA intersectiontargets into the project box, organisms select "Homo sapiens", other parameters unchanged,and then get the drug and disease PPI network data, and finally the results will be exported in TSV format. Use Cytoscape 3.9.0 software to plot the mesh and analyze key targets based on degree values.

1.6 GO and KEGG enrichment analysis

Using the DAVID online data platform (https://david.ncifcrf.gov/), select the "Shortcut to DAVID Tools" project bar, import the intersection targets of TwHF and RA into it, set the threshold to $p < 0.05$, select the "Select Identifier" column (offcial genne symbol), and select "Homo" in the "Select species" column sapiens", functional enrichment of Genes Ontology (GO) and pathway analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG).

1.7 Molecular Docking

First find the ligand, use the TCMSP database to export the 2D structure of the TwHF core target, enter the pubchem web page (https://pubchem.ncbi.nlm.nih.gov/) to convert the 2D structure into a 2D structure in "SDF" format, and use Chem3D software to convert the 2D structure into a 3D structure; Then find the receptor, import the core target into the UniProt database, download the protein molecular data in the format of "PDB" through the PDB database (https://www.rcsb.org/), use PyMOL software to "dehydrate" and "remove impurities" of the PDB protein molecules, and finally obtain the receptor; Finally, the collected ligands and receptors were introduced into AutoDock Tools software for processing, and the 3D binding diagram was drawn using PyMOL software.

2 Results

2.1 Screening of active ingredients and targets of TwHF

A total of 54 active ingredients were obtained by searching the TCMSP database, and the active ingredient screening conditions of $30\% \leq OB$ and $0.18 \leq DL$ were used, and finally 7 TwHF active ingredients were screened out (see Table 1). The results of the selected active ingredients were compiled, and the target gene matching was performed on the Uniprot data platform to remove duplicate target proteins and non-human target proteins, and finally 155 potential targets were obtained.

2.2 RA disease target screening

The RA targets obtained from OMIM, GeneCards, TTD, pharmGKB, drugbank and other databases are 41, 4882, 177, 9, 595, and a total of 4917 RA targets were obtained after merging and removing duplicates. The collated TwHF targets and RA targets were mapped in Venn 2.1.0 website, and then Venn diagram was drawn (Fig. 2). A total of 87 intersection targets were obtained from the Venn diagram, and the top 15 intersection targets are listed below (Table 2).

Table 2
Intersection of active ingredient of TwHF genes and RA genes (before 15)

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

2.3 "Drug component-target-disease" network diagram

The 87 intersecting targets from the Venn diagram were used to create a "drug component-target gene-disease" network by Cytoscape 3.9.0 software(Fig. 3). Based on this network topology, the top 10 genes were plotted in a histogram using the degree value as a reference standard(Fig. 4). The compounds with higher degree values were microRNA 132 (MIR-132), peptidyl arginine deaminase-4 (PAD-I4), and microRNA 155 (MIR-155), indicating that TwHF may treat RA synergistically through the above 3 components.

2.4 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 by String online platform and Cytoscape 3.9.0 software(Fig. 5 and Fig. 6).

the top 3 targets in terms of degree, namely tumor necrosis factor (TNF), p53 tumor protein (TP53), and vascular endothelial growth factor-A (VEGFA).

2.5 GO function and KEGG enrichment analysis

The 87 intersection targets filtered were imported into the DAVID database for G0 function and KEGG enrichment analysis. GO function is mainly divided into three aspects: cellular component (CC), molecular function (MF), biological process (BP), etc,of which BP includes kidney development, positive regulation of positive regulation of calcineurin-NFAT signaling calcineurin-NFAT signaling cascade), positive regulation of endocytosis, etc;MF includes G protein β-subunit binding, transcription regulatory region sequence-specific DNA binding, kinase activity, etc;CC includes the nucleus, the perinuclear region of cytoplasm, and the early endosome(Fig. 7). KeGG pathway enrichment analysis obtained nuclear factor kappa-B (NF-κB) signaling pathway, Toll-like receptor (TLRs) signaling pathway, Vascular endothelial growth factors (VEGF) signaling pathway, etc(Fig. 8).

2.6 Molecular docking

The active ingredients micro-ribonucleic acid 132, peptide arginine deimidase 4, and micro-ribonucleic acid 155, which are in the top three of the "active ingredient-target" network diagram, were connected to the key targets TNF, TP53 and VEGFA obtained by topology analysis, and the output of the results was optimized by 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[7] .The results showed that there may be 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(Fig. 9).

3 Discussion

RA is a symmetric, inflammatory peripheral polyarthritis of unknown etiology, which has a high incidence, recurrence, and disability rate[8],Epidemiologicalsurveys show that the prevalence of RA is 0.24% globally, 0.3-1% in developed countries[9],[and 0.28–0.45% in China[10].The pathogenesis of RA remains unclear,and some studies suggest that cytokines, signaling pathways, lymphocytes, and other A variety of factors contribute to the development and expression of the disease[11].Early treatment and attainment therapy are currently the main treatment strategies for RA, with complete remission or low disease activity as the treatment goal[12,13].The current treatment of RA is mainly based on modern drug therapy, but these drugs require long-term use and some may be resistant[14].Therefore, the search for new anti-RA drugs is one of the urgent problems that researchers need to solve,and 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.TwHF was first recorded in the book Dian Nan Ben Cao written by Lan Mao in the Ming Dynasty, which says that it is pungent in taste, warm in nature and poisonous. It enters the twelve meridians of the liver and spleen and travels through the twelve meridians. So far, more than 100 kinds of ingredients such as alkaloids, diterpenoids, triterpenoids and polysaccharideshave been isolated from Ligustrum[15,16].In

recent years, studies have confirmed the immunosuppressive, anti-inflammatory, antioxidant, analgesic and neuroprotective effects of various components of TwHF[17–19],but the potential mechanisms by which the active components of TwHF improve the physicochemical indices of RA are not clear enough to systematically elaborate their biological processes and metabolic pathways.

Network pharmacology provides a new strategy to study Chinese medicine from a multi-target approach[20].In this study, the "drug component-target-disease" network diagram revealed that the active components of TwHF in the treatment of RA were mainly MIR-132, PAD-l4 and MIR-155. Among them, miRNA is an endogenous non-coding small RNA, which is involved in the pathogenesis of the body's disease by regulating the transcription and translation of mRNA, and plays an important regulatory role in the various stages of immune cellmaturation and maintenance of immune homeostasis, and is closely related to the development of autoimmune diseases[21–25].In addition, miRNAs can play an important rolein the pathophysiology of RA patients by regulating matrixmetal-oproteinases (MMPs)[26].Chatzikyriakidou A found that miR-132 plays an important role in the pathophysiology of peripheral blood mononuclear cells (PBCs) in RA patients[27].The expression of miR-132 was found to be upregulated in peripheral blood mononuclear cells (PBMC) of RA patients,and the polymorphism of its target gene was closely related to the pathogenesis of RA, suggesting thatmiR-132 could be a molecular marker of RA disease activity[28].Elmesmarifound

that miR-155 was highly expressed in peripheral blood and monocytes of RA patientsand could promote the accumulation of inflammatory cells in the synovial membrane through theregulation of chemokine and pro-inflammatory chemokine receptor expression, triggering the disease[29].Alivernini found that miR-155 ishighly expressed in peripheral blood B cells of RA patients, which can promote the expression of inflammatory factors such asIL-21 in synovial tissue, whichis related to the severity of inflammation in synovial tissue, indicating that miR-155 is an important regulator of activation of B cells in RA disease[30].In addition, studies have shown that upregulation of serum exosome miR-155 expressionmay be involved in the development of RA disease,which provides ideas for

further studyof how serum exosomes can participate in regulating the occurrence and development of RA disease[31].PAD-l4 is an important modified enzyme after protein translation, and is a member of the PAD family, which is mainly distributed in the cytoplasm of macrophages,granulocytes, and monocytes, and can catalyze the conversion of arginine into citrulline residues[32].There is a close relationship between PAD-l4 activity disorders and the development of RA disease, and studies have shown that the susceptibility gene of RA is associatedwith PAD-l4, which is expressed in blood and RA synovial tissue[33].

According to the analysis of PPI network diagram, the top 3 targets of degree valuesare 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, proliferation, and angiogenesis[34].VEGF-A 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[35]. Studies have shownthatVEGF-A can not only promote the formation of RA synovial vascular fender, but alsoone 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[36]. VEGF-A interacts with the inflammatory cytokines of RA, interacts with each other, 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,and can act on synovial cells, macrophages, chondrocytes, and osteoclasts in RA joint tissue, and activate these cells, leading to local inflammatory responsesand vascular formation, causing cartilage destruction and bone erosion[37].At present, biologics represented by TNF inhibitors can effectively improve the condition of RA, and many clinical trialshave confirmed that TNF inhibitors can improve joint inflammation and joint function, reduce theclinical activity of RA, and delay the radiological progression of joints [38–40].The TP53 gene is an oncosmopressor gene, the TP53 gene encodes the P53 protein, and its main role is to

regulate the cell cycle, repair damaged DNA, induce apoptosis, and inhibit angiogenesis[41].Studies have shown that the P53 gene affects apoptosis of RA synovial fibroblasts in ratmodels of adjuvant arthritis (AA) and may alleviate diseaseprogression if the P53 gene isoverexpressed early in the disease[42].Based on theabove results, it can be seen that there is a synergistic effect between the active ingredients of TwHF, and may play a role in treating RA by reducing the level of inflammatory transmitters and regulating immunity.

GO functional enrichment analysis showed that TwHF in the treatment of RA BP mainly involved kidney development, positive regulation of calcineurin-NFAT signal cascade, positive regulation of endophagy, etc., MF mainly involved G protein β subunit binding, transcriptional regulatory region sequence specific DNA binding, kinase, etc.KEGG enrichment analysis shows that the signal paths involved are NF-κB signal path, TLRs signal path, VEGF signal path, etc.It is believed that NF-κB is an important downstream protein of multiple signaling pathways, which is involved in the transcription process of cytokines, adhesion molecules, and proteases that regulate a variety of inflammatory responses, and is highly activated in the RA synovium, inducing the secretion of a variety of pro-inflammatory factors, resulting in synovitis and joint destruction[43].It has been found that TwHF can inhibit 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 RA patients[44,45].The TLRs signaling pathway plays an important role in the progression of the course of RA, and the absence of a death domainof myeloid differentiation factor 88 (MyD88) in the TLRs signaling pathway can lead to limited recruitment activity of downstream interleukin (IL)-1 receptor-associated kinases, weakening the role of IL-6, IL-12, and TNF-α[46].In addition, TwHF extracts have been found to reduce the production of inflammatory transmitters and reduce inflammation by inhibiting signaling factor expression in the TLR4/NF-κB signaling pathway[47].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[48].

In summary, this study applied network pharmacology and molecular docking methodsto conduct exploratory studies on the active ingredients, targets and signaling pathways of TwHF in the treatment of RA, and found that a variety of active ingredients in TwHF can participate in the positive regulation of endophagy, G protein β subunit binding, transcriptional regulatory region sequence-specific DNA binding, kinases, etc., and comprehensively targeted regulation through NF-κB signaling pathway, TLRs signaling pathway, VEGF signaling pathway, etc., so as to play a therapeutic role.This study preliminarily explores themechanism of action of TwHF in the treatment of RA, and also provides new ideas and references for the in-depth study of TwHF.

Declarations

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Competing interests

None.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data avaliability statement

Data openly available in a public repository,Through the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http: //old.tcmsp-e.com/tcmsp.php) ,we searched for "Lei Gong Deng ", obtain all chemical components of TwHF.In the OOMI database

(https://www.omim.org), GeneCards database (https://www.genecards.org), TTD database (https://db.idrblab.net/ttd), pharmGKB database (https://www.pharmgkb.org), In the drugbank database (https://go.drugbank.com) obtain all "rheumatoid arthritis" targets.

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Table

Table 1 is available in the Supplementary Files section

Figures

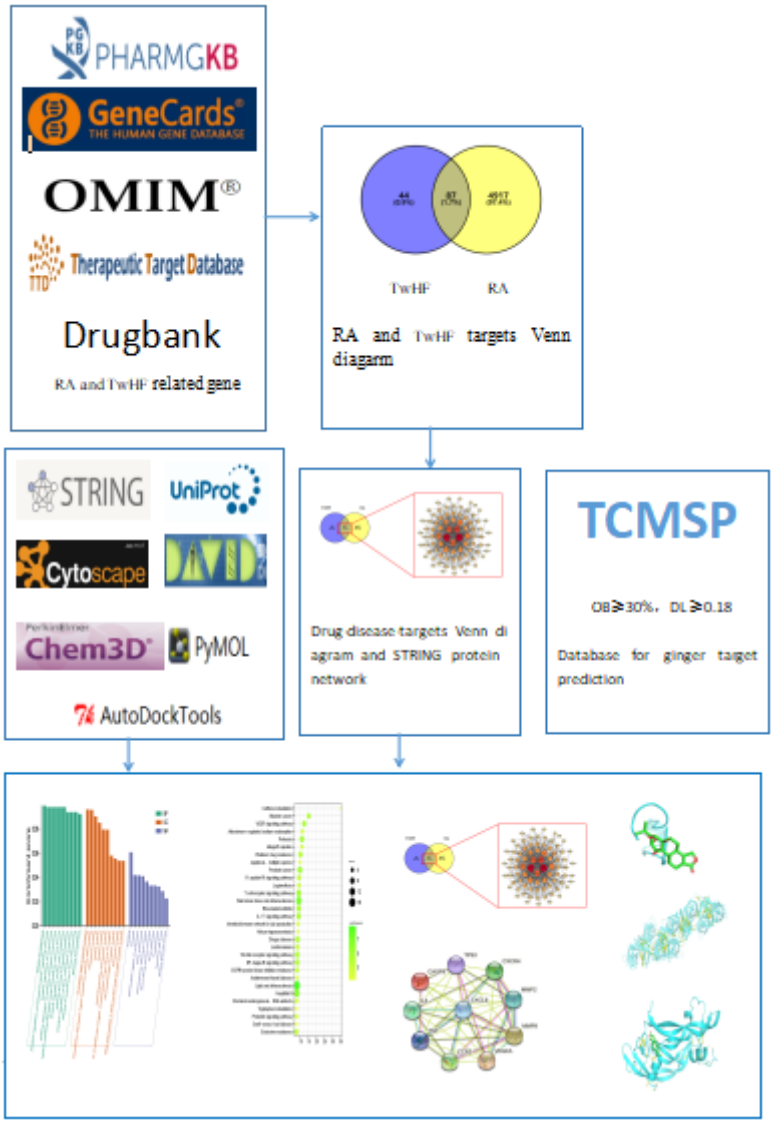


Figure 1

Workflow of the study.The underlying mechanism of TwHFagainst RA would be investigated using network pharmacology and molecular docking.gastric mucosal injury, RA, rheumatoid arthritis; GO,Gene Ontology;KEGG,Kyoto.Encyclopedia of Genes and Genomes.

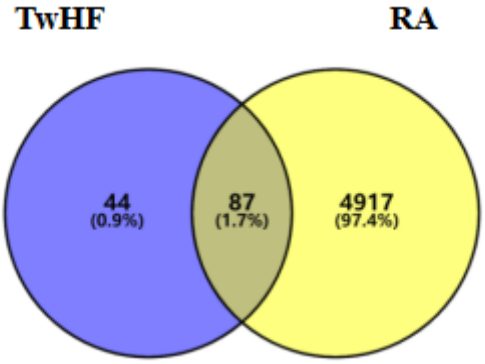


Figure 2

Venn diagram of active ingredient of TwHF and RA intersection targets

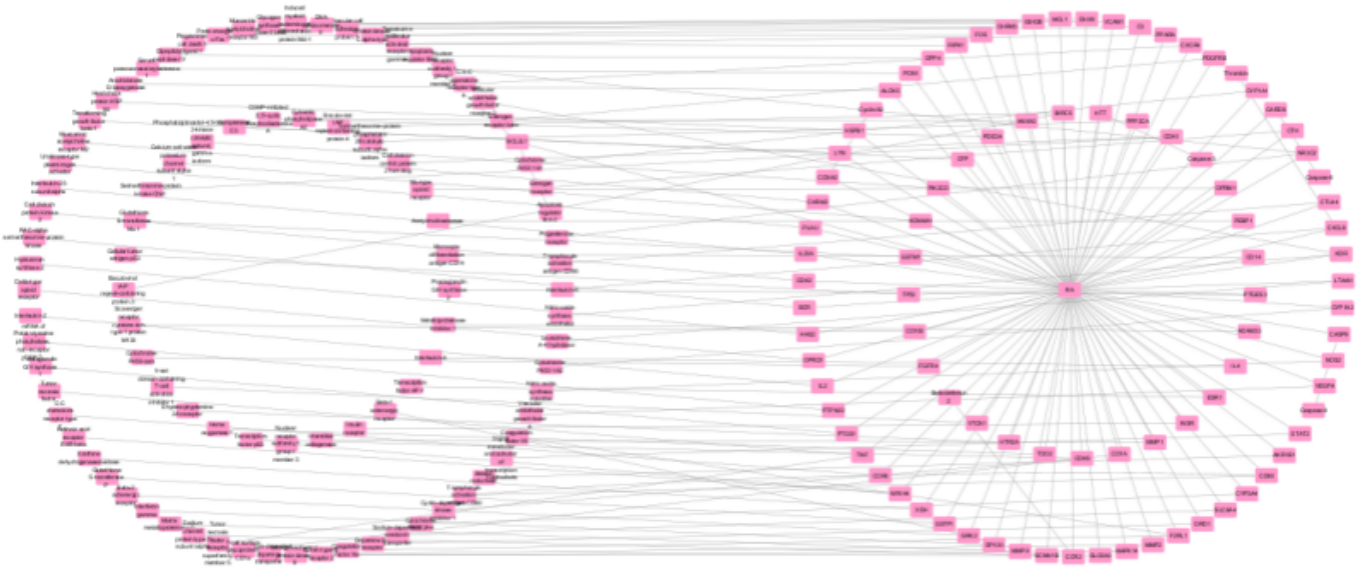


Figure 3

"Active ingredient-target-RA" network

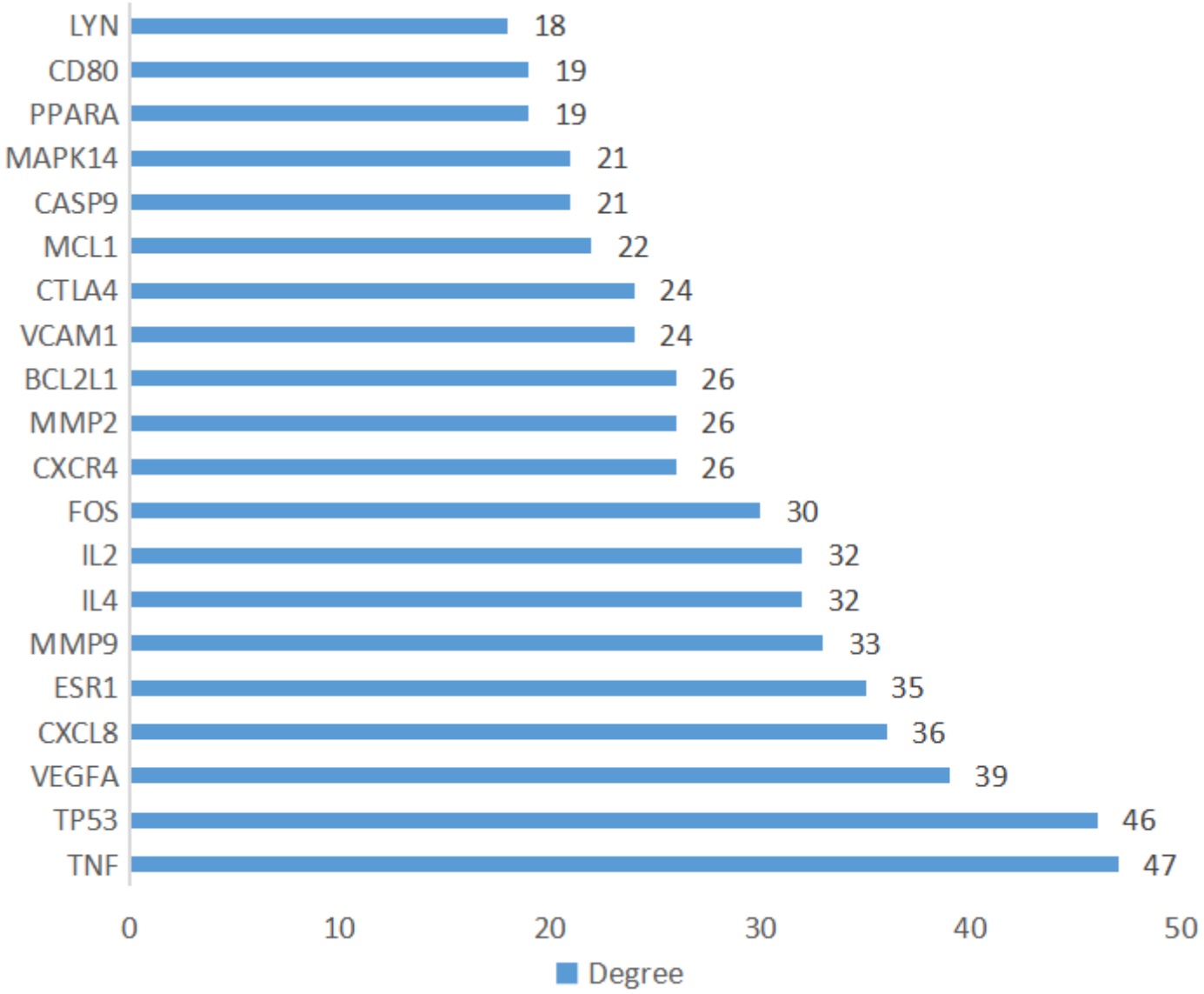


Figure 4

Top 20 gene histogram

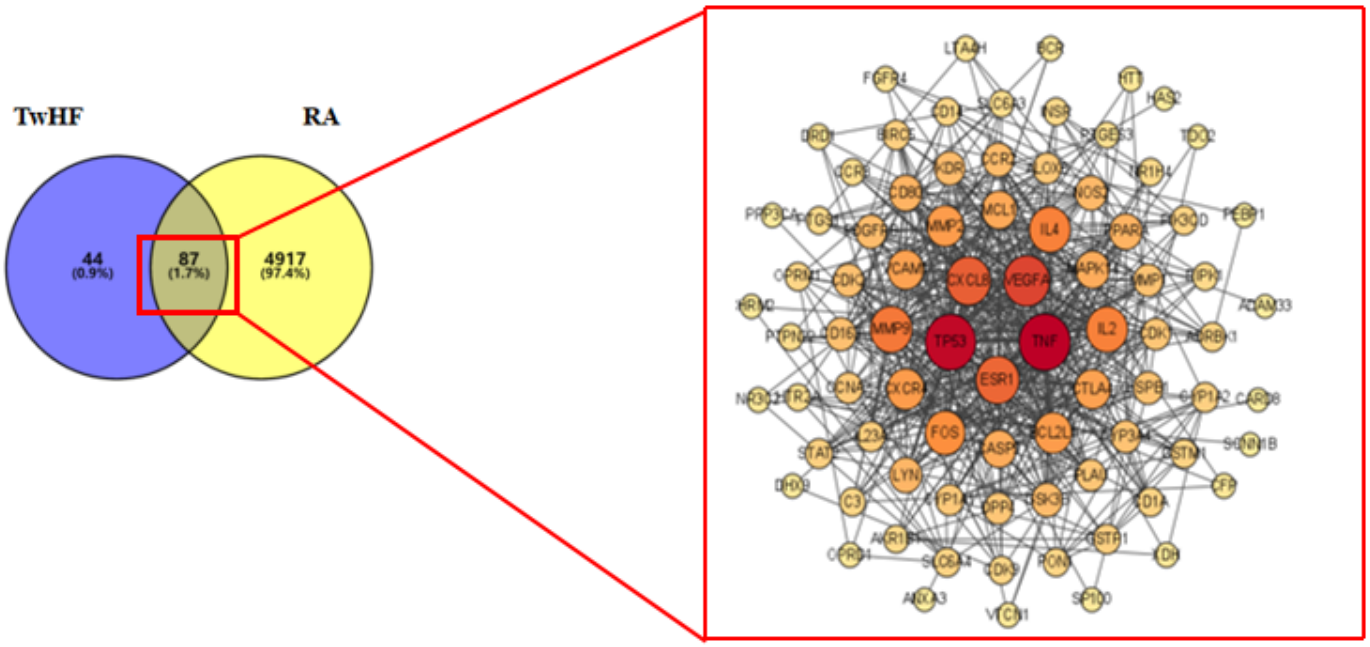


Figure 5

PPI network diagram

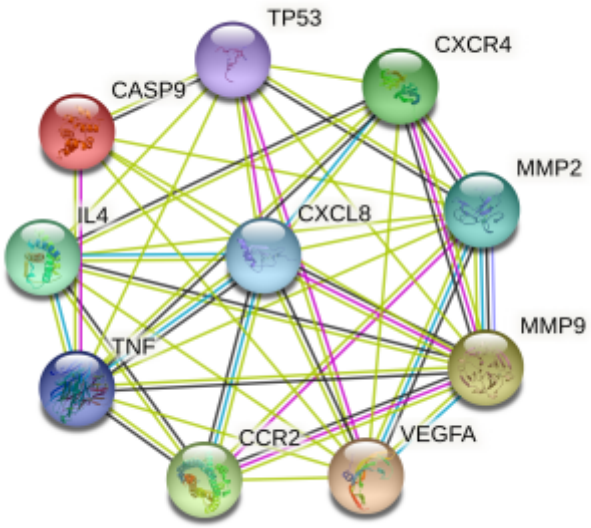


Figure 6

Key proteins in PPI

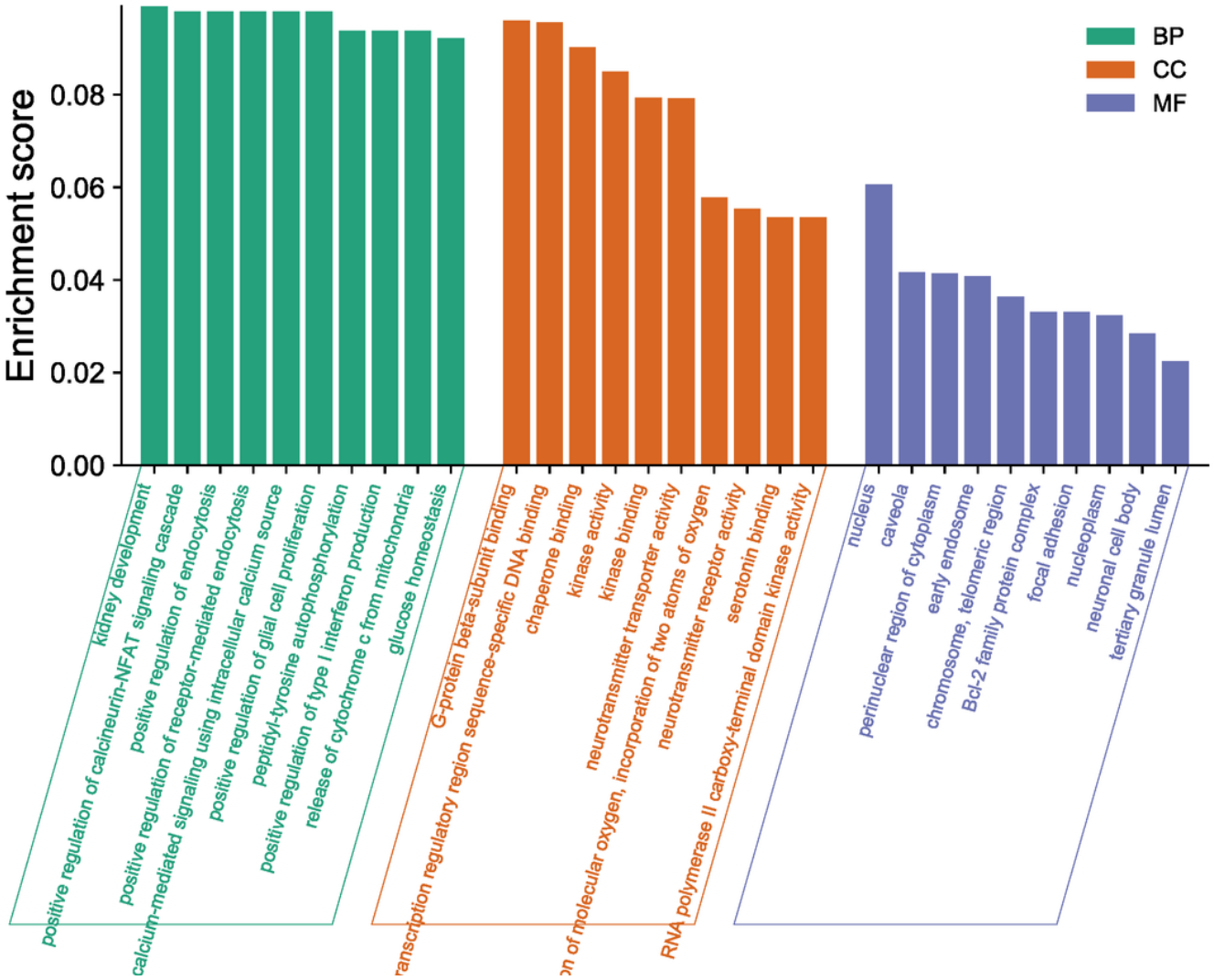


Figure 7

GO function enrichment analysis

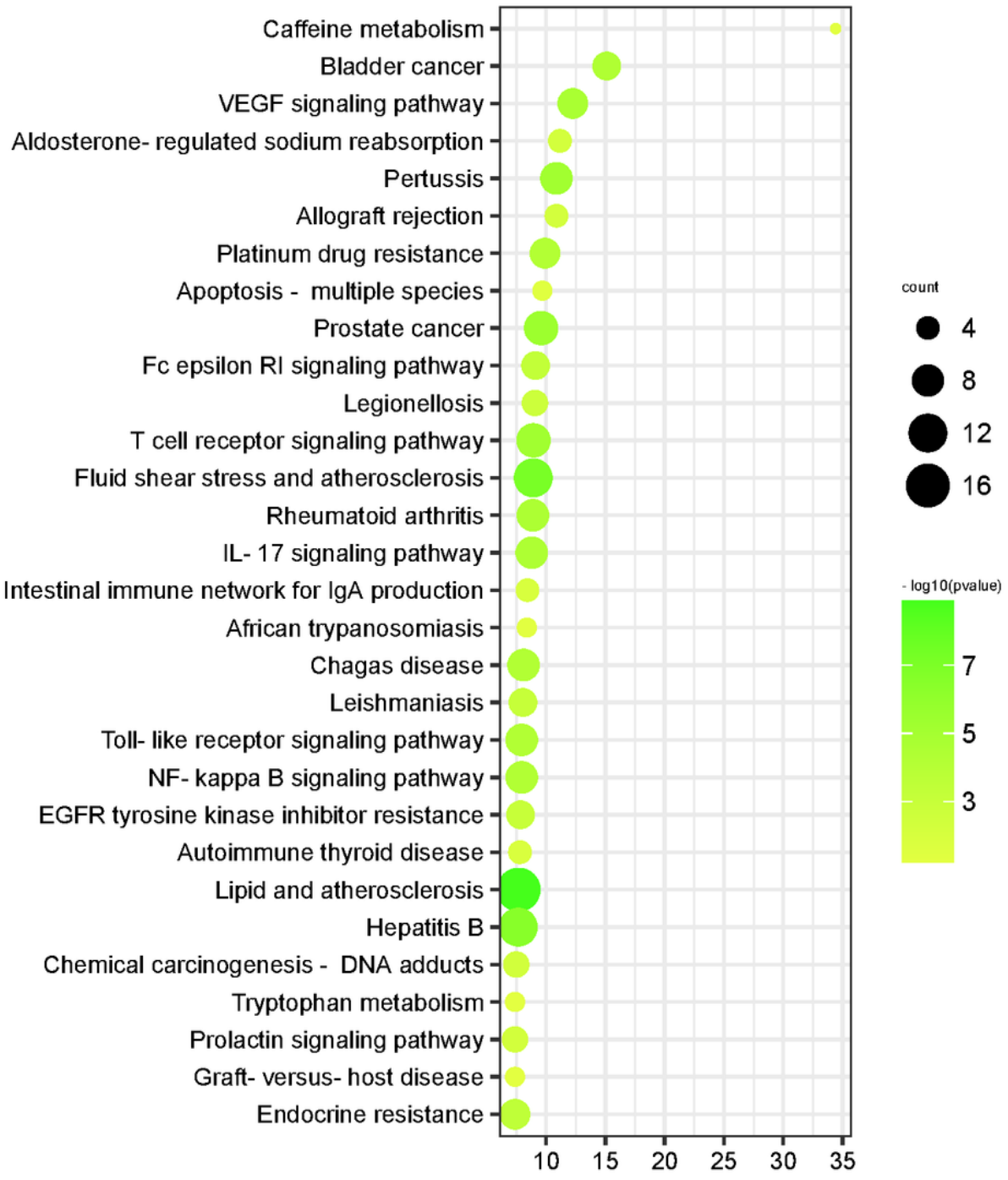


Figure 8

KEGG pathway enrichment analysis

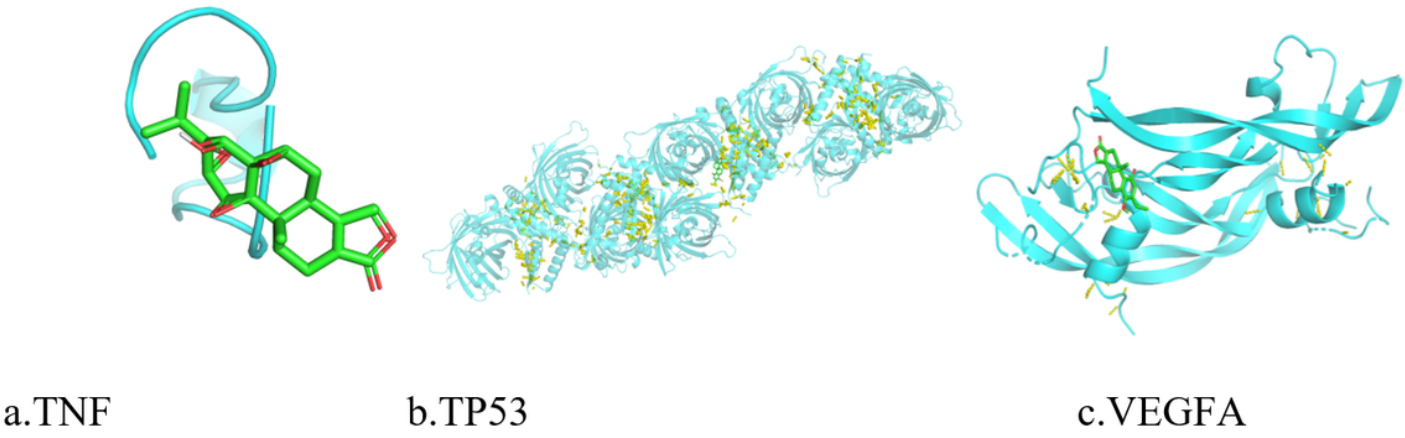


Figure 9

Molecular docking of active components of TwHF and key target

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1ActiveingredientsofTwHF.docx