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Original Article

Systems pharmacology-based study of the molecular mechanism of triptolide for treating rheumatoid arthritis

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Abstract: We conducted a systems pharmacology-based study on the molecular mechanism of triptolide for treating rheumatoid arthritis (RA). The absorption, distribution, metabolism, excretion, and toxicity profile of triptolide were assessed using the Traditional Chinese Medicine Systems Pharmacology database and the SwissADME, and ACD/Labs software packages. DRAR-CPI, PharmMapper, similarity ensemble approach, and SwissTargetPrediction were used to predict the potential targets of triptolide. RA-related target genes were identified from the DisGeNET, GeneCards, Online Mendelian Inheritance in Man, and DrugBank databases, and the differentially expressed genes in RA were identified from the Gene Expression Omnibus database to obtain the potential targets genes in the use of triptolide for treating RA. The DAVID 6.8 was used for the enrichment analysis of potential target genes by using the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes databases. Finally, macromolecular docking of the targets of triptolide and identical targets of Western medications reported by the DrugBank database were compared using AutoDock Vina. Twenty potential target genes of triptolide for treating RA were identified. Triptolide was found to have 28 biological functions and 18 related pathways, respectively. Macromolecular docking verified that triptolide has excellent binding affinity with its targets and has superior binding score to Western medications. Our findings improve the understanding of the molecular mechanism of triptolide and provide a powerful method for modern Chinese medicine research.

Keywords: Triptolide, systems pharmacology, ADMET, effect and mechanism, macromolecular docking

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease targeting mainly the synovial membrane. RA initially presents as synovitis, followed by articular cartilage damage, and can involve other connective tissues including pericardium and pleura. In the early stages, medicines may help alleviate the clinical symptoms. In later stages, most patients exhibit joint destruction and deformation and may even develop permanent disability, which severely affects their quality of life and life expectancy [1]. The medicines most commonly used for alleviating the inflammation and retarding disease progression in RA are methotrexate, prednisolone, and infliximab. However, their long-term use can have adverse effects on the kidney, liver, digestive tract, and respiratory tract [2]. Therefore, other effective alternatives are needed.

Traditional Chinese medicine has long been used to treat RA. It has excellent treatment outcomes, is safe, and causes few adverse effects [3]. *Tripterygium wilfordii* Hook. F. (TWHF) is a traditional Chinese medicine that is used to relieve pain, reduce swelling, detoxify, and kill worms. This medicine is often employed clinically to treat primary and secondary glomerulonephritis, chronic glomerulonephritis, nephrotic syndrome, anaphylactoid purpura nephritis, and RA [4]. Its main active ingredient is triptolide, which is derived from its root. Triptolide is a terpene with multiple biological activities, including antioxidant, anti-RA, anti-Alzheimer's disease, and anticancer effects. It

is the main effective component of *Tripterygium wilfordii* tablets and Tripterygium glycoside tablets.

Studies on triptolide use in the treatment of RA have focused on clinical applications, pharmacological activity testing, and drug safety [5]. Only a few reports have assessed the molecular mechanisms underlying its effects in the treatment of RA from the perspective of systems biology [6]. Therefore, in this study, we used systems pharmacology [7] (i.e., cheminformatics, bioinformatics, and network pharmacology) to perform in-depth analysis of the molecular mechanisms underlying the effects of triptolide in the treatment of RA and provide a theoretical foundation for further study and clinical application.

Our previous studies successfully predicted the active compounds and molecular targets of several Chinese herbal formulas, including the Yinchenhao decoction [8], Danlu capsule [9], Xuangui dropping pill [10], Bo-ai capsule [11], Zuojin pill [12], and Huanglian Jiedu decoction [13]. A detailed workflow of the systems pharmacology used in this study is presented in **Figure 1**.

Materials and methods

Absorption, distribution, metabolism, excretion, and toxicity profile of triptolide

The canonical simplified molecular-input line-entry system and spatial data file (SDF) were downloaded from the PubChem platform (https://pubchem.ncbi.nlm.nih.gov/) [14]. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile of triptolide was identified using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://lsp.nwu.edu.cn/tcmsp.php) [15], and the SwissADME (http://www.swissadme.ch/) [16] and Advanced Chemistry Development (ACD/Labs) (https://www.acdlabs.com/) software packages. The molecular weight, oral bioavailability (OB), drug-likeness (DL), intestinal epithelial permeability (Caco-2), and blood-brain barrier (BBB) information of triptolide was retrieved from the TCMSP database. Furthermore, its gastrointestinal (GI) absorption and bioavailability scores were estimated using SwissADME. Moreover, Lipinski's rule of five, metabolic stability, Ames test, and human ether-a-go-go-related gene (hERG) for triptolide were generated using ACD/Labs.

Collection and compilation of the target data on RA-related genes

RA-related genes were identified from the DisGeNET (http://disgenet.org/home/) [17], GeneCards (https://www.genecards.org/) [18], and Online Mendelian Inheritance in Man (OMIM) (https://omim.org/) databases [19] as follows: The keyword "Rheumatoid Arthritis" was entered to search for potential targets in RA-related genes, and all genes with a gene-disease association score higher than the average score were included. Next, all genes identified from the three databases were combined to obtain a final dataset of RA-related genes. In addition, established drugs used to treat RA were identified from the DrugBank database (https://www.drugbank.ca/) [20], and their corresponding target data were collected (**Figure 2**).

Selection of differentially expressed genes in RA from the gene expression omnibus database

To comprehensively examine the data on the potential target genes in RA, differentially expressed genes were identified from the Gene Expression Omnibus (GEO) database and selected for analysis by using the three gene chips (GSE55235, GSE55457, and GSE77298) downloaded from the database (https://www.ncbi.nlm.nih.gov/geo/) [21]. Sample data of healthy individuals and patients with RA were drawn from 27 and 39 samples, respectively. Subsequently, the differentially expressed genes were analyzed using the limma package. In addition, RA-related genes were identified according to logFC > 1.0, logFC < -1.0, or adj.P.Val < 0.05.

We selected differentially expressed genes that overlapped in two or more datasets as potential targets in RA-related genes. Next, the data of the differentially expressed genes obtained from the GEO database and target data collected from DrugBank were combined. The intersection method was used to identify all RA-related genes from the DisGeNET, GeneCards, and OMIM database and examine whether the associated proteins were related to RA. Thus, data on RA-related gene targets were obtained.

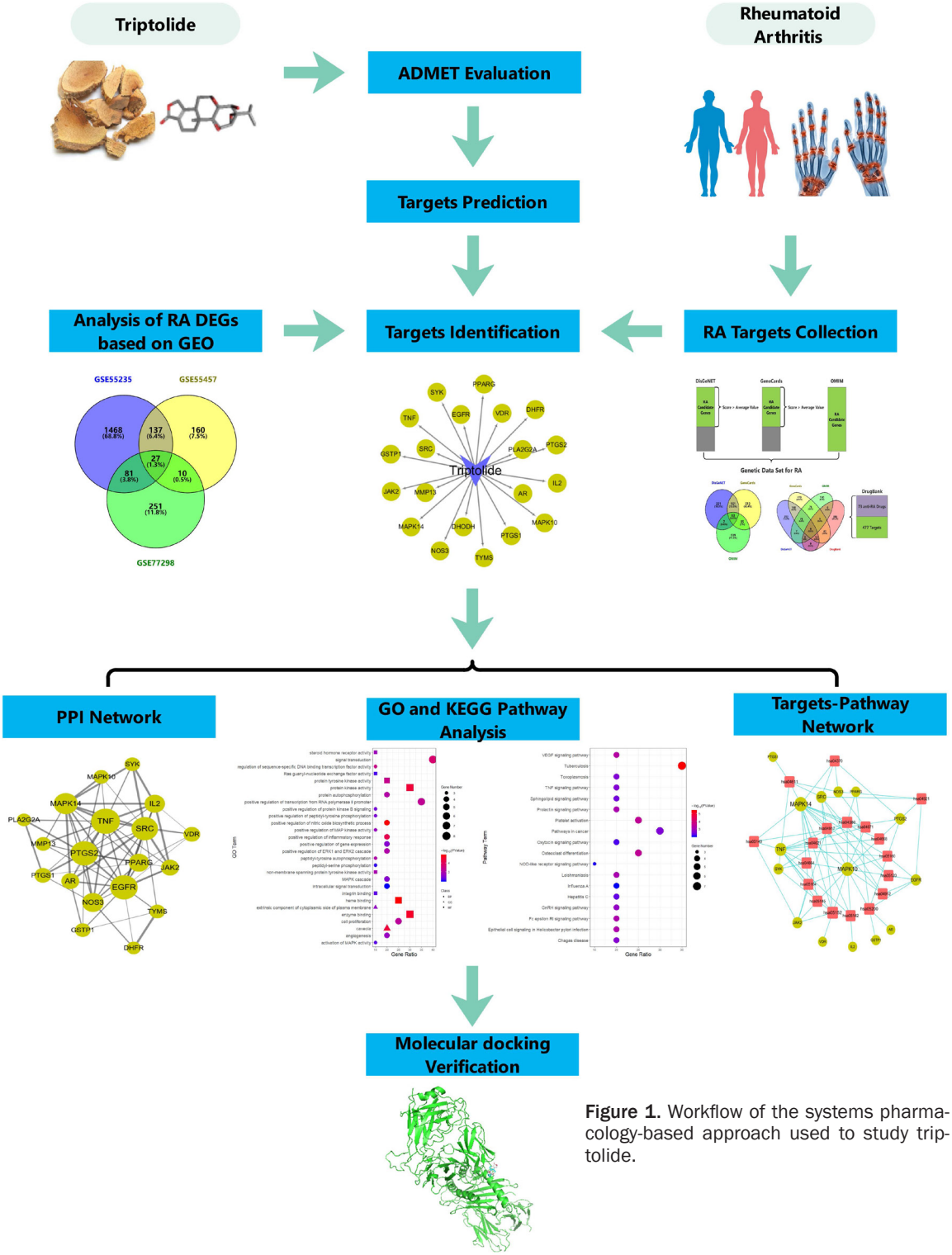

Figure 1. Workflow of the systems pharmacology-based approach used to study triptolide.

Prediction and identification of the potential targets of triptolide for treating RA

Four databases-Drug Repositioning and Adverse Drug Reaction via Chemical-Protein Interactome (DRAR-CPI) (http://cpi.bio-x.cn/drar/) [22], PharmMapper (http://lilab-ecust.cn/pharmmapper/) [23], Similarity ensemble approach (SEA) (http://sea.bkslab.org/) [24], and SwissTargetPrediction (http://swisstargetprediction.ch/) [25] were used to predict, combine, and compile the potential targets of trip-

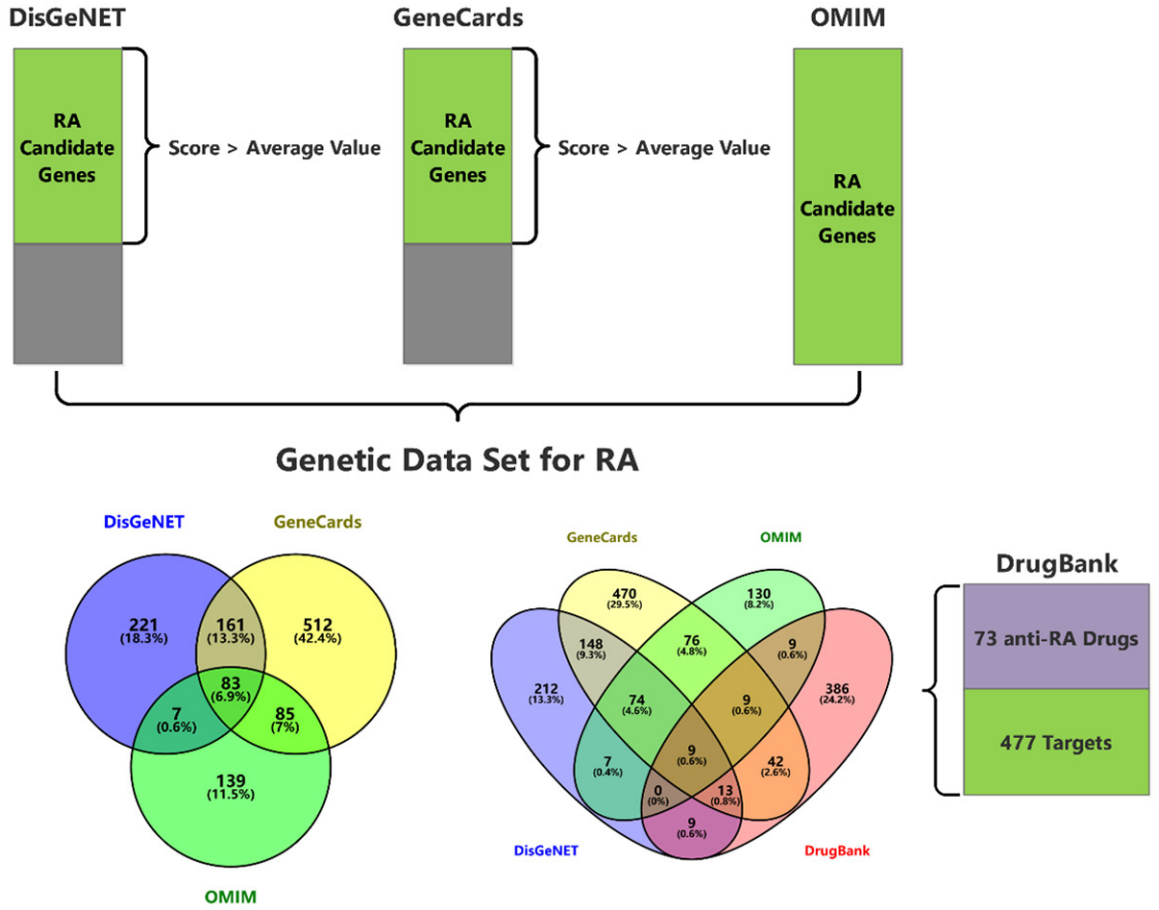

Figure 2. Collection and compilation of RA-related genes and the drugs used in RA with their corresponding target data.

tolide. The predicted targets and obtained target data on RA-related genes were matched to identify the target proteins of triptolide for the treatment of RA. These target proteins were input to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (https://string-db.org/) [26] to construct an interactive network of the target proteins of triptolide in RA treatment. "Homo Sapiens" was used as the "Organism" and the "Interaction Score" was set at 0.4. Cytoscape 3.7.1 (https://cytoscape.org/) [27] was used to draw the targets of triptolide and the corresponding protein-protein interaction (PPI) network diagram.

Gene ontology and the kyoto encyclopedia of genes and genomes enrichment analysis for the targets of triptolide

To further analyze the molecular mechanism of the potential targets of triptolide, the Database for Annotation, Visualization, and Integrated Discovery (DAVID) 6.8 (https://david.ncifcrf.gov/) [28] was used for enrichment analysis employing the Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases. DAVID 6.8 provides comprehensive and systematic information about the biological functions of numerous genes and proteins; therefore, interpretations of enriched biological function can be obtained from this database. The enrichment statistical analysis method comprised the hypergeometric test and Fisher exact test; we employed p value < 0.01 in DAVID 6.8. Additionally, according to the interaction between triptolide and its protein targets as well as the GO and KEGG enrichment analysis, a data file was established. Cytoscape version 3.7.1 was used to construct a network diagram of the targets of triptolide and its pathways.

Table 1. ADMET prediction of triptolide

ADMET evaluation items	Letter abbreviation	Prediction results	Source of method
Molecular weight	MW	360.40 g/mol	TCMSP
Oral bioavailability (%)	OB (%)	51.29	
Drug-likeness	DL	0.68	
Caco-2 permeability	Caco-2	0.25	
Blood-brain barrier	BBB	-0.19	
Intestinal absorption rate	GI absorption	High	SwissADME
Bioavailability Score	BAS	0.55	
Lipinski five rules	Ro5	Good	ACD/Labs
Metabolic stability	MS	Undefined	
Mutation assay	Ames	Mutagenic	
Cardiotoxicity	hERG	Undefined	

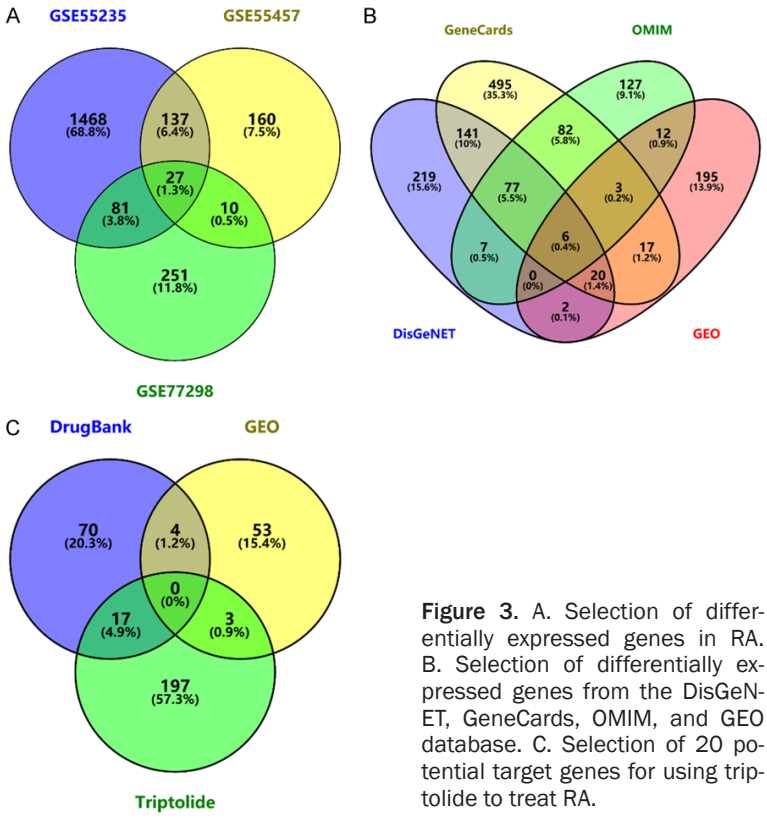

Figure 3. A. Selection of differentially expressed genes in RA. B. Selection of differentially expressed genes from the DisGeNET, GeneCards, OMIM, and GEO database. C. Selection of 20 potential target genes for using triptolide to treat RA.

Macromolecular docking of the targets of triptolide

To examine the binding affinity between triptolide and its potential targets and compare these targets with the identical targets in Western medications, the DrugBank database was downloaded from the PubChem platform (https://pubchem.ncbi.nlm.nih.gov/), and the SDF file for the 3D-structures of the drugs for RA was obtained from the DrugBank database. In addition, files on the protein structure of the targets of triptolide were collected from the Protein Data Bank (PDB) (http://www.rcsb.org/) [29], by using UniProt ID. The PyMOL software (https://pymol.org/) [30] was used to preprocess all the small molecules and target proteins and produce a PDB file. Subsequently, the target proteins were input to POCASA version 1.1 software (http://altair.sci.hokudai.ac.jp/g6/service/pocasa/) [31] to calculate the optimal binding site for each of the ligands of the target proteins. Next, the AutoDock Vina software package (http://vina.scripps.edu/) [32] was used for semi-flexible refinement in macromolecular docking to calculate the binding scores of all the small molecules and their targets. Furthermore, these results were compared with the macromolecular-docking results for the targets of triptolide in the drugs provided by the DrugBank database. The Wilcox. test function of R language (v. 3.5.1) was used to perform the Wilcoxon rank-sum test on the basis of the results of molecular docking, and a two-sided test was adopted.

Results

Assessment of the ADMET profile of triptolide

The TCMSP database, and SwissADME, and ACD/Labs software packages were used to systematically determine the ADMET profile of triptolide. OB, DL, GI absorption, bioavailability score, and Lipinski's rule of five indicated that triptolide possesses excellent medicinal properties. However, ACD/Labs software indicated that triptolide may have mutagenic toxicity,

Table 2. Twenty potential targets of triptolide in the treatment of RA

Gene	Protein Name	Uniprot ID	Degree
AR	Androgen receptor	P10275	6
DHFR	Dihydrofolate reductase	P00374	3
DHODH	Dihydroorotate dehydrogenase (quinone)	Q02127	0
EGFR	Epidermal growth factor receptor	P00533	14
GSTP1	Glutathione S-transferase P	P09211	4
IL2	Interleukin-2	P60568	8
JAK2	Tyrosine-protein kinase JAK2	O60674	6
MAPK10	Mitogen-activated protein kinase 10	P53779	4
MAPK14	Mitogen-activated protein kinase 14	Q16539	11
MMP13	Collagenase 3	P45452	4
NOS3	Nitric oxide synthase	P29474	9
PLA2G2A	Phosphatidylcholine 2-acylhydrolase 2A	P14555	3
PPARG	Peroxisome proliferator-activated receptor gamma	P37231	8
PTGS1	Prostaglandin G/H synthase 1	P23219	5
PTGS2	Prostaglandin G/H synthase 2	P35354	14
SRC	Proto-oncogene tyrosine-protein kinase Src	P12931	13
SYK	Tyrosine-protein kinase SYK	P43405	4
TNF	Tumor necrosis factor	P01375	15
TYMS	Thymidylate synthase	P04818	4
VDR	Vitamin D3 receptor	P48281	5

which is consistent with the reports on the adverse effect of TWHF. Nevertheless, adverse effects related to metabolic stability and cardiotoxicity were not observed clearly. Therefore, further evidence should be collected from in vitro and in vivo experiments. The data on the ADMET profile of triptolide are presented in **Table 1**.

Collection and compilation of target data on RA-related genes

472, 841, and 314 RA-related genes were obtained from the DisGeNET, GeneCards, and OMIM database, respectively. After combining these three databases, we identified 1208 RA-related genes. From the DrugBank, 73 confirmative drugs for RA were collected and 477 targets were identified. The intersection of the targets corresponding to these drugs was collected from the DrugBank database. The RA-related genes obtained from the three gene databases indicated that 91 targets for Western medications were identified as target proteins in RA (**Figure 2**).

Selection from the GEO database of differentially expressed genes in RA

Three chips (GSE55235, GSE55457, and GSE77298) were analyzed, leading to 255 differentially expressed genes being obtained from patients with RA and healthy individuals (**Figure 3A**). The intersection of the 225 differentially expressed genes and the three RA-related genes from the databases indicated that 60 of 255 differentially expressed genes were pathogenic RA-related genes (**Figure 3B**).

Prediction and identification of the potential target genes of triptolide for treating RA

After the SwissTargetPrediction, SEA, PharmMapper, and DRAR-CPI databases were used to predict the potential targets of triptolide. The results were combined to obtain 217 potential target genes, which were then intersected with the RA-related genes obtained from the DrugBank and GEO database (**Figure 3C**). We discovered that triptolide potentially targets 20 anti-RA genes (**Table 2**). These 20 genes were introduced to the STRING database to construct an interactive network of proteins. Cytoscape 3.7.1 was used to construct the network of target genes and PPI network of triptolide (**Figure 4A**, **4B**). In the PPI network, the sizes and colors of the nodes were used to reflect the "degree". Through network analysis, we found that TNF (degree = 15), PTGS2 (degree = 14), EGFR (degree = 14), SRC (degree = 13), and MAPK14 (degree = 11) with high degree values are the hub nodes of the PPI network; in other words, they are targeted by triptolide, and thus have biological significance.

GO and KEGG enrichment analysis of the potential target genes of triptolide

DAVID 6.8 was used for GO and the KEGG enrichment analysis of the 20 potential target genes ($P < 0.05$; **Table 3**). **Figure 5A** and **5B** present the results of the enrichment analysis.

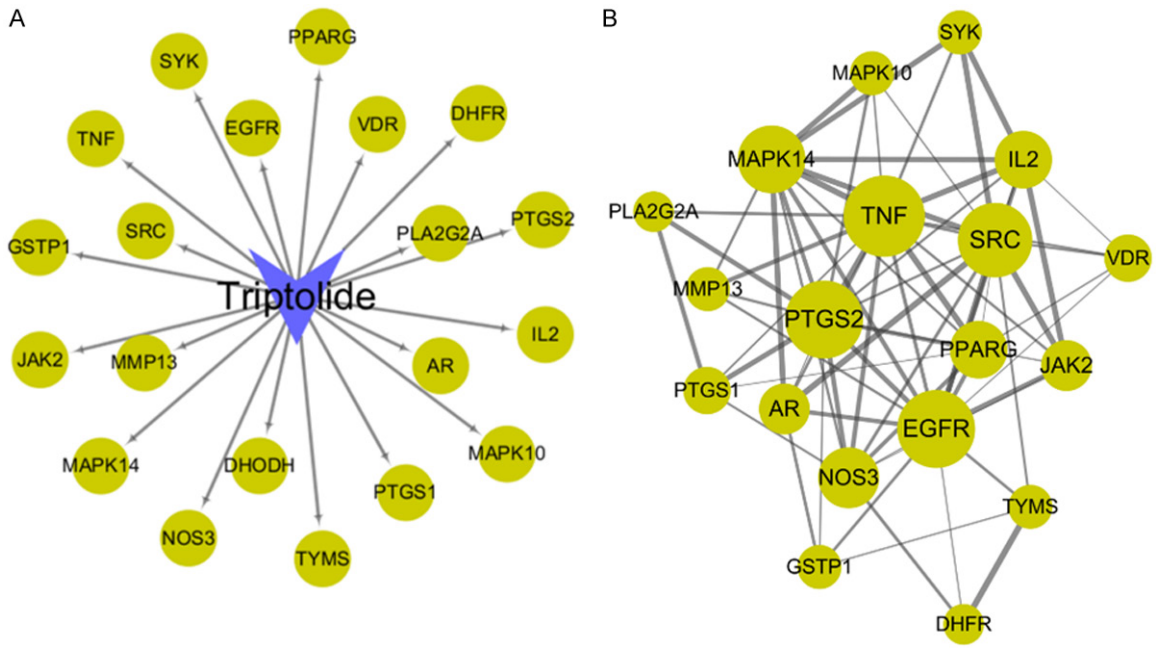

Figure 4. A. Network of the potential target genes of triptolide. B. Interactive network of 20 potential target genes in RA. The node size represents the magnitude of the degree, and the thickness of the edge represents the size of the combined score.

Table 3. GO and KEGG enrichment analysis of the target genes of triptolide in RA

ID	Pathway Name	Count	Genes
hsa05152	Tuberculosis	7	MAPK14, JAK2, MAPK10, SRC, SYK, TNF, VDR
hsa05200	Pathways in cancer	6	EGFR, GSTP1, AR, PPARG, MAPK10, PTGS2
hsa04611	Platelet activation	5	MAPK14, NOS3, PTGS1, SRC, SYK
hsa04380	Osteoclast differentiation	5	MAPK14, PPARG, MAPK10, SYK, TNF
hsa04370	VEGF signaling pathway	4	MAPK14, NOS3, PTGS2, SRC
hsa04921	Oxytocin signaling pathway	4	EGFR, NOS3, PTGS2, SRC
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	4	MAPK14, EGFR, MAPK10, SRC
hsa04664	Fc epsilon RI signaling pathway	4	MAPK14, MAPK10, SYK, TNF
hsa04912	GnRH signaling pathway	4	MAPK14, EGFR, MAPK10, SRC
hsa04917	Prolactin signaling pathway	4	MAPK14, JAK2, MAPK10, SRC
hsa05140	Leishmaniasis	4	MAPK14, JAK2, PTGS2, TNF
hsa05142	Chagas disease	4	MAPK14, IL2, MAPK10, TNF
hsa04668	TNF signaling pathway	4	MAPK14, MAPK10, PTGS2, TNF
hsa05145	Toxoplasmosis	4	MAPK14, JAK2, MAPK10, TNF
hsa04071	Sphingolipid signaling pathway	4	MAPK14, NOS3, MAPK10, TNF
hsa05160	Hepatitis C	4	MAPK14, EGFR, MAPK10, TNF
hsa05164	Influenza A	4	MAPK14, JAK2, MAPK10, TNF
hsa04621	NOD-like receptor signaling pathway	3	MAPK14, MAPK10, TNF

GO enrichment analysis entails a directed acyclic graph comprising a certain number of proteins or genes at a functional level. This graph features the main biological processes, molecular functions, and cellular components. According to the results of the present enriched analysis, the number of enrichment target genes exceeded five, and the genes mainly involved signal transduction (GO: 0007165), regulation of for RNA polymerase II promoter transcription (GO: 0045944), enzyme binding (GO: 0019899), protein kinase activity (GO:

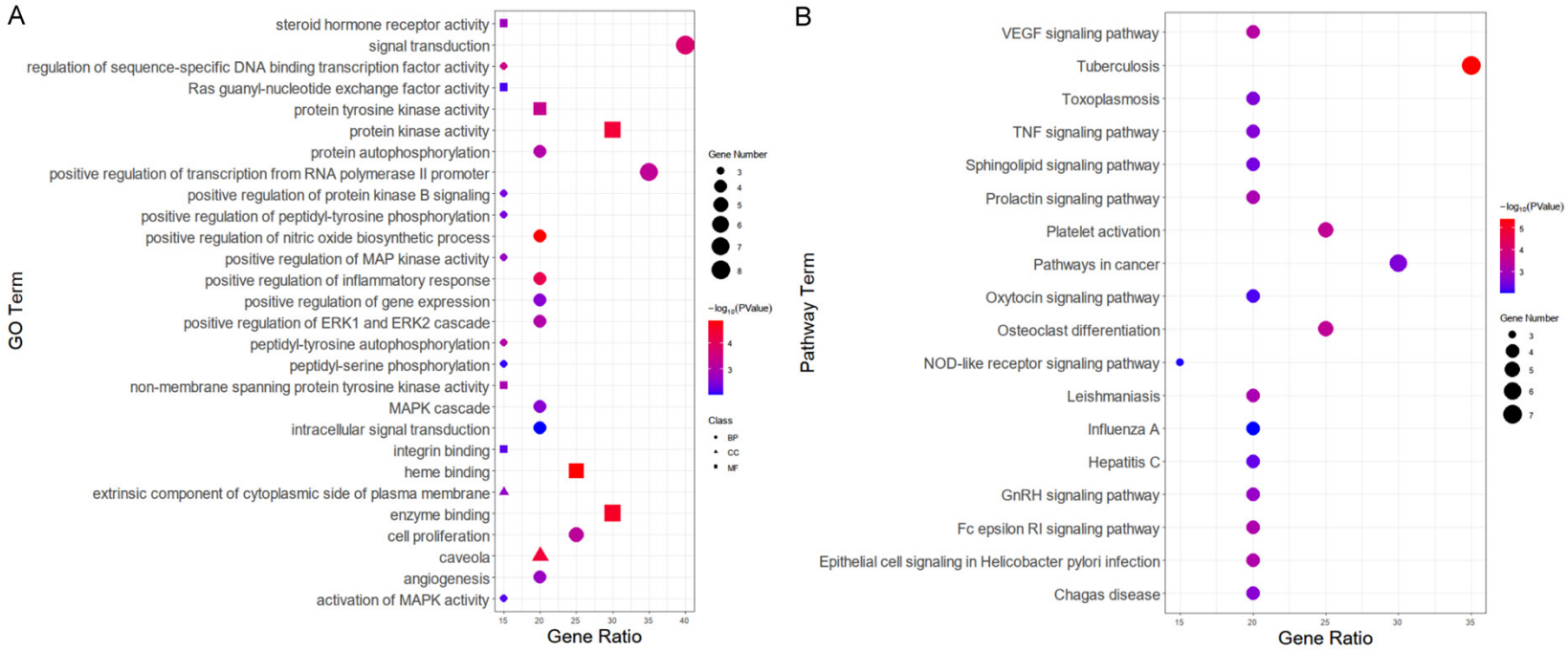

Figure 5. (A) GO and (B) KEGG enrichment analyses of the 20 potential target genes of triptolide in RA.

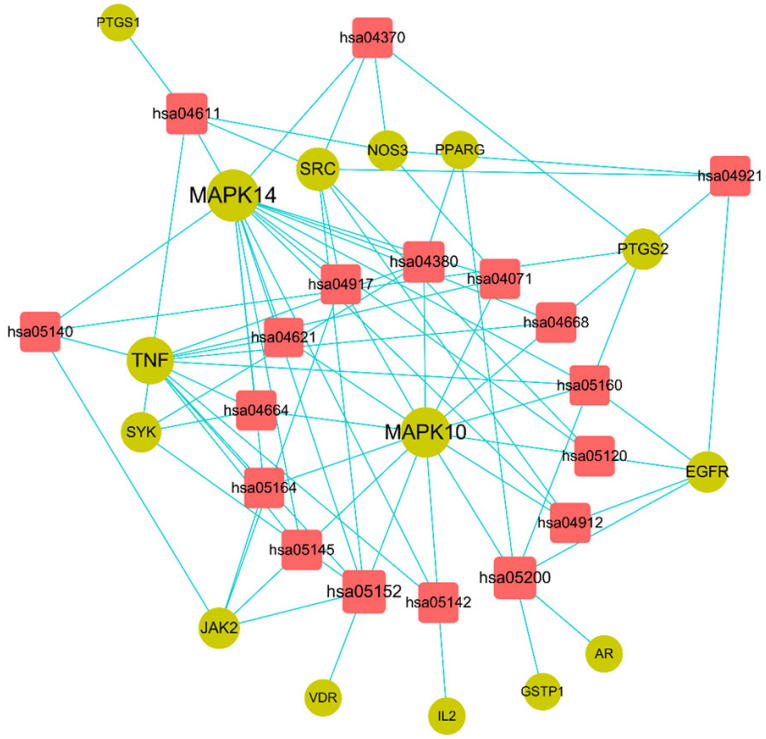

Figure 6. Target-pathway network of differentially expressed genes from the triptolide.

0004672), cell proliferation (GO: 0008283), and hemoglobin binding (GO: 0020037).

KEGG enrichment analysis revealed that the target genes of triptolide influence the following pathways: signaling in cancer (hsa05200), platelet activation (hsa04611), osteoclast differentiation (hsa04380), vascular endothelial growth factor (VEGF) signaling (hsa04370), and tumor-necrosis factor (TNF) signaling (hsa04668). According to the pathway enrichment analysis of the target genes, an anti-RA target-pathway network for triptolide was obtained (**Figure 6**). **Figure 6** reveals that *MAPK14, MAPK10, and TNF* are closely related to other pathways and are crucial target molecules of triptolide for the treatment of RA.

Macromolecular docking of the targets of triptolide and western medications

The binding scores between triptolide and the following target proteins were lower than -5.0 (indicating excellent binding affinity): DHODH, EGFR. IL2, JAK2, MAPK10, MAPK14, NOS3, PTGS1, PTGS2, SRC, SYK, and TNF. In addition, for MAPK14, NOS3, and SYK, the binding scores were lower than -9.0, implying that these proteins may be the key targets of triptolide for the treatment of RA. As presented in **Table 4**, the binding scores were generally lower than those of Western medications reported in the DrugBank database (a lower score is preferred), indicating that triptolide is superior to Western medications (e.g., acemetacin, apremilast, and fostamatinib) in terms of its effects on the potential target genes in RA.

Discussion

More than 100 chemical components have been identified in TWHF. Among these, triptolide is the most effective, as well as the most studied one. It has anti-inflammatory, analgesic, antitumor, and immunomodulatory effects and is especially effective in the treatment of RA [33].

In this study, first, the ADMET profile of triptolide indicted that triptolide has excellent pharmacological activity. Next, we used ACD/Labs software to predict the toxicity of triptolide, and found that triptolide has mutagenicity. This result is consistent with other reports indicating that triptolide in tripterygium glycosides is the main component of TWHF that causes nephrotoxicity [34].

Subsequently, by collecting target data on RA-related genes and analyzing the GEO chip data, we identified 20 potential targets of triptolide for the treatment of RA. These targets were introduced into the STRING database to construct a PPI network. Through network analysis, we discovered that TNF, PTGS2, MAPK14, EGFR, and SRC are hub genes with a high degree of connectivity and have high value in research on RA treatment.

Next, DAVID 6.8 was employed for GO and KEGG enrichment analysis. According to the analysis, triptolide has effects in RA through signal transduction, positive regulation of RNA polymerase II promoter transcription, enzyme binding, protein kinase activity, and cell proliferation. Triptolide can be used to treat RA by

Table 4. Macromolecular docking of the key targets of triptolide and Western medications provided by the DrugBank database

Ligand	Receptor	PDB ID	Affinity	Ligand	Receptor	PDB ID	Affinity
Aceclofenac	PTGS1	3MV7	-7.5	Meclofenamic acid	PTGS1	3MV7	-8.9
Aceclofenac	PTGS2	5IKV	-7.3	Meclofenamic acid	PTGS2	5IKV	-8.3
Acemetacin	PTGS1	3MV7	-6.1	Mefenamic acid	PTGS1	3MV7	-7.8
Acemetacin	PTGS2	5IKV	-5.9	Mefenamic acid	PTGS2	5IKV	-7.2
Acetaminophen	PTGS1	3MV7	-7.7	Meloxicam	PTGS1	3MV7	-7.3
Acetaminophen	PTGS2	5IKV	-8.1	Meloxicam	PTGS2	5IKV	-7.6
Alclofenac	PTGS2	5IKV	-9.1	Menthyl salicylate	PTGS1	3MV7	-7.9
Apremilast	IL2	3QB1	-6.1	Menthyl salicylate	PTGS2	5IKV	-7.1
	NOS3	1M9J	-4.4	Nabumetone	PTGS1	3MV7	-7.8
Apremilast	TNF	2AZ5	-4.9	Nabumetone	PTGS2	5IKV	-8.3
Aspirin	PTGS1	3MV7	-7.7	Naproxen	PTGS1	3MV7	-5.9
Aspirin	PTGS2	5IKV	-8.1	Naproxen	PTGS2	5IKV	-7.5
Baricitinib	JAK2	3TJC	-7.0	Oxaprozin	PTGS1	3MV7	-8.1
Celecoxib	PTGS1	3MV7	-8.5	Oxaprozin	PTGS2	5IKV	-9.0
Celecoxib	PTGS2	5IKV	-8.3	Phenylbutazone	PTGS1	3MV7	-9.1
Chloroquine	TNF	2AZ5	-7.7	Phenylbutazone	PTGS2	5IKV	-8.1
Choline magnesium trisalicylate	PTGS1	3MV7	-7.4	Piroxicam	PTGS1	3MV7	-7.7
Choline magnesium trisalicylate	PTGS2	5IKV	-7.7	Piroxicam	PTGS2	5IKV	-8.2
Diclofenac	PTGS1	3MV7	-7.4	Rofecoxib	PTGS2	5IKV	-8.3
Diclofenac	PTGS2	5IKV	-8.1	Salsalate	PTGS1	3MV7	-9.1
Diflunisal	PTGS1	3MV7	-7.8	Salsalate	PTGS2	5IKV	-9.7
Diflunisal	PTGS2	5IKV	-7.1	Sulfasalazine	PTGS1	3MV7	-6.4
Etodolac	PTGS1	3MV7	-8.1	Sulfasalazine	PTGS2	5IKV	-7.9
Etodolac	PTGS2	5IKV	-7.2	Sulindac	PTGS1	3MV7	-8.5
Etoricoxib	PTGS2	5IKV	-7.7	Sulindac	PTGS2	5IKV	-8.1
Fenoprofen	PTGS1	3MV7	-7.8	Tenoxicam	PTGS1	3MV7	-7.1
Fenoprofen	PTGS2	5IKV	-6.6	Tenoxicam	PTGS2	5IKV	-7.4
Flurbiprofen	PTGS1	3MV7	-7.8	Tiaprofenic acid	PTGS1	3MV7	-7.6
Flurbiprofen	PTGS2	5IKV	-8.1	Tiaprofenic acid	PTGS2	5IKV	-7.3
Fostamatinib	EGFR	5ZT0	-5.0	Tofacitinib	JAK2	3TJC	-7.7
Fostamatinib	JAK2	3TJC	-7.0	Tolmetin	PTGS1	3MV7	-4.4
Fostamatinib	MAPK10	3CGF	-6.4	Tolmetin	PTGS2	5IKV	-4.9
Fostamatinib	MAPK14	6HWT	-7.8	Triptolide	DHODH	6QU7	-8.7
Fostamatinib	SRC	1YI6	-9.1	Triptolide	EGFR	5ZT0	-8.7
Fostamatinib	SYK	3SRV	-8.2	Triptolide	IL2	3QB1	-7.5
Glucosamine	TNF	2AZ5	-8.0	Triptolide	JAK2	3TJC	-8.8
Ibuprofen	PTGS1	3MV7	-8.1	Triptolide	MAPK10	3CGF	-7.5
Ibuprofen	PTGS2	5IKV	-7.7	Triptolide	MAPK14	6HWT	-9.1
Indometacin	PTGS1	3MV7	-8.6	Triptolide	NOS3	1M9J	-9.0
Indometacin	PTGS2	5IKV	-5.7	Triptolide	PTGS1	3MV7	-8.6
Ketoprofen	PTGS1	3MV7	-8.0	Triptolide	PTGS2	5IKV	-8.3
Ketoprofen	PTGS2	5IKV	-7.4	Triptolide	SRC	1YI6	-7.8
Ketorolac	PTGS1	3MV7	-8.2	Triptolide	SYK	3SRV	-9.0
Ketorolac	PTGS2	5IKV	-7.5	Triptolide	TNF	2AZ5	-8.7
Leflunomide	DHODH	6QU7	-9.2	Trolamine salicylate	PTGS1	3MV7	-7.7
Lidocaine	EGFR	5ZT0	-6.6	Trolamine salicylate	PTGS2	5IKV	-7.2

producing pharmacological effects, such as platelet activation pathway (hsa04611), VEGF signaling pathway (hsa04370) [35], and TNF signaling pathway (hsa04668) [36]. The effects of triptolide on RA include inhibition of the production of proinflammatory cytokines and inflammatory mediators, influencing the proliferation of T lymphocytes, inhibiting angiogenesis, and inducing apoptosis [37]. For example, reports have indicated that the proinflammatory cytokine TNF-α is a critical factor causing chronic inflammation related to RA [38]. Excessive TNF-α not only causes abnormalities in its signaling pathway but also works with other proinflammatory cytokines to stimulate osteoclast differentiation and activity, thereby inducing bone loss. This results in persistent recruitment, activation, retention, and survival of immunocytes in the synovium of patients with RA, leading to the destruction of joints and cartilage or even more severe outcomes. Triptolide inhibits proinflammatory cytokines and reduces osteoclast differentiation and activity by regulating TNF, PTGS2, MAPK14, MAPK10, and NOS3 in the TNF signaling pathway, thereby achieving symptom control with favorable treatment effects in RA.

Finally, AutoDock Vina was used for macromolecular docking of the targets of triptolide and to compare the binding scores of target of triptolide with those of the identical targets of Western medications provided by the DrugBank database. The results revealed that triptolide has high binding affinity; its binding score was superior to that of the Western medications, which confirmed that triptolide can be used to treat RA effectively.

In summary, adopting the systems pharmacology approach led to comprehensive and profound understanding of the molecular mechanism of triptolide. Furthermore, the approach provides a foundation for researching and optimizing the effects of triptolide, indicating that it is a novel research method and perspective in Chinese medicine research and development.

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Disclosure of conflict of interest

None.

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