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Network pharmacology, molecular docking and bioinformatics reveal the mechanism of Tripterygii Wilfordii against Osteosarcoma

Yafang Zhang, MD[a], Junqiang Wei, MD[a], Lingwei Kong, MD[a], Mingze Song, MD[a], Yange Zhang, MD[a], Xiangyu Xiao, MD[a], Haiying Cao, MD[a], Yu Jin, PhD[a,*]

Abstract

Osteosarcoma (OS) is a malignant bone tumor of mesenchymal origin. Tripterygii Wilfordii (TW) is a traditional Chinese medicine widely used for its anti-inflammatory and immunomodulatory effects. Various components of TW have been shown to have antitumor effects, however, no systematic study has been conducted to prove the anti-OS effects of TW. This study aimed to investigate the effects of TW on OS and its mechanism based on network pharmacology and molecular docking. The web pharmacology section includes the gathering of the active components of TW, the collection of predicted targets of TW and OS-related targets, the analysis of therapeutic targets of TW, the enrichment of gene ontology (GO), and the enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG). The Veen diagram showed 451 targets for OS treatment in TW. The therapeutic target enrichment analysis results showed that TW treated OS via multiple targets and pathways. TW can affect OS proliferation, apoptosis, migration, infiltration, and angiogenesis through a signaling network formed by hub genes that cascade through numerous signaling pathways. In addition, molecular docking results showed that triptolide, kaempferol, and 5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin have relatively high potential to become drugs for patients with OS and improve the 5-year survival rate of patients with OS. Network pharmacology and molecular docking suggest that TW affects the biological behavior of OS through multiple pathways involving multiple targets, such as proliferation, apoptosis, migration, and infiltration. Upregulation of the cellular tumor antigen p53 (TP53) gene and downregulation of peroxisome proliferator-activated receptor gamma (PPARG) and signal transducer and activator of transcription 1-alpha/beta (STAT1) genes can prolong the survival time of patients with OS. Triptolide, kaempferol, and 5,8-Dihydroxy-7-(4-hydroxy-5 methyl-coumarin-3)-coumarin have a relatively high potential to become a treatment for patients with OS and improve 5-year survival of OS patients.

Abbreviations: ETCM = the encyclopedia of traditional Chinese medicine, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, KM = Kaplan Meier, OS = osteosarcoma, PPARG = peroxisome proliferator-activated receptor gamma, STAT1 = signal transducer and activator of transcription 1-alpha/beta, PPI = protein-protein interaction networks, RELA = transcription factor p65, TCMSP = the traditional Chinese medicine system pharmacology database, and analysis platform, TNF = tumor necrosis factor, TP53 = cellular tumor antigen p53, TW = Tripterygii Wilfordii.

Keywords: mechanism, molecular docking, network pharmacology, osteosarcoma, Tripterygii Wilfordii

1. Introduction

Osteosarcoma (OS) is the most prevalent type of bone.[1] It occurs most frequently in the epiphyses of weight-bearing long stem bone,[2] such as the distal femur and proximal tibia.[3] OS occurs in 2 to 3/million/year and is more prevalent in adolescents,[4,5] at 8 to 11/million/year.[4] Current clinical treatment options have include neoadjuvant chemotherapy, radical resection, and adjuvant chemotherapy. The 5-year survival rate for patients with in situ OS is 65% to 70%[6]; the 5-year survival rate for patients with distant metastases and recurrent OS is 10% to 40%.[6] Although new treatments are constantly being attempted, the 5-year survival rate has not changed for decades.[7] There is an urgent need to find new treatments to increase the overall survival of patients with OS.

Tripterygii Wilfordii (TW) is the traditional Chinese medicines.[8] As early as hundreds of years ago, TW has been used in herbal prescriptions to treat various diseases.[8] In

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The datasets generated during and/or analyzed during the current study are publicly available.

The original contributions presented in the study are included in the Material and Methods, and further inquiries can be directed to the corresponding authors.

Because we used public and anonymous data, according to the ethics guidelines, neither informed consent nor approval from the ethics committee was required.

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[a] *Department of Traumatology and Orthopaedics, Affiliated Hospital of Chengde Medical College, Chengde, Hebei, China.*

**Correspondence: Yu Jin, Department of Traumatology and Orthopaedics, Affiliated Hospital of Chengde Medical College, Chengde 067000, China (e-mail: jinyucdyxyfy@126.com).*

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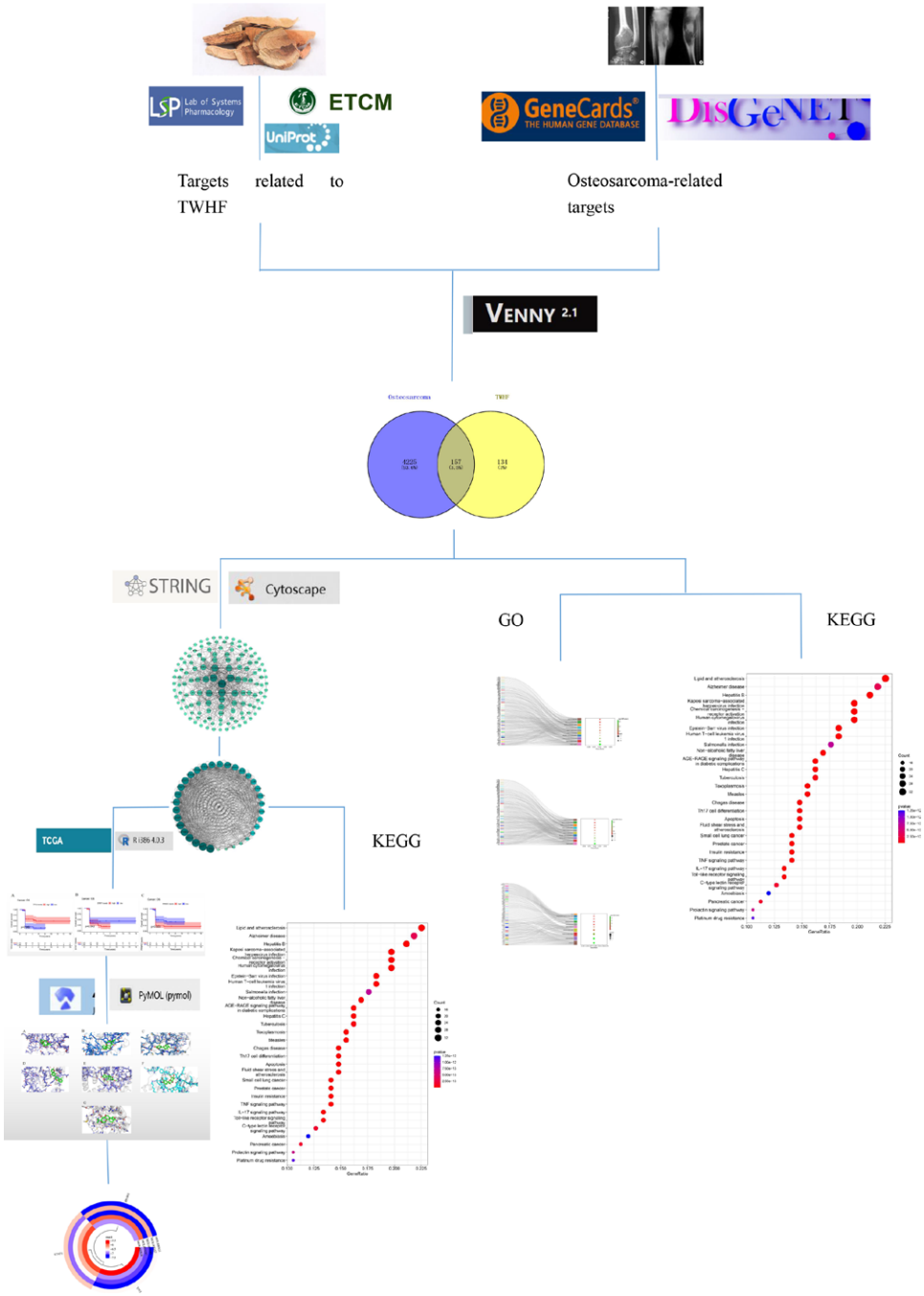

Figure 1. Network pharmacological study of Tripterygii Wilfordii for the treatment of osteosarcoma schematic diagram.

modern pharmacology, numerous reports have demonstrated the anti-inflammatory and immunomodulatory functions of TW.[9] Meanwhile, many components of TW, such as triptolide, celastrol, and tripchlorolide, have been shown to mediate tumor cell apoptosis and inhibit tumor angiogenesis and radiosensitize.[10–13] For example, triptolide inhibits IDH1 mutated malignancies via Nrf2-driven glutathione metabolism.[10] Triptolide induces apoptosis in cervical cancer by inactivating RAC-alpha serine/threonine-protein kinase.[14] Celastrol has been reported in publications to have cytotoxic, anti-apoptotic, anti-angiogenic, and radiosensitizing effects on many tumor cells.[11,13,15] However, there is still a lack of literature on whether TW affects OS and its impact mechanism.

As a discipline combining system biology, bioinformatics, and high throughput histology,[16] network pharmacology is increasingly used in the field of pharmacology, especially in discovering herbal medicines.[17,18] In this study, we used network pharmacology to predict whether TW can affect OS and the possible mechanism of its effect on OS. In addition, we used a molecular docking technique to predict the active components of TW that have relatively large effects on OS. A flowchart of the research performed in this study is shown in Figure 1.

2. Materials and Methods

2.1. Obtain the targets related to TW

In the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP) database（https://old.tcmsp-e.com/tcmsp.php）, The keyword "TW" was used to search for the active ingredients of TW and the targets related to TW. The obtained active ingredients were screened

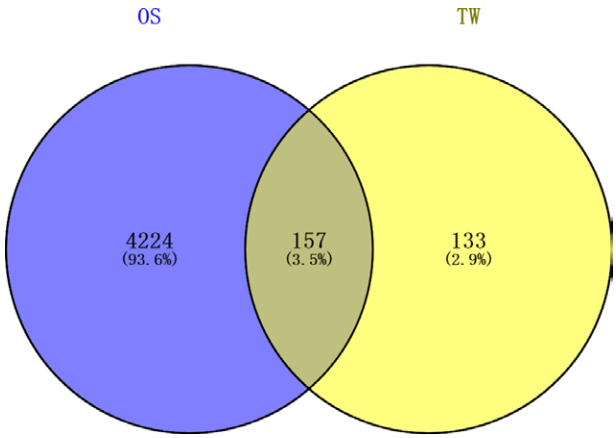

Figure 2. The veen diagram about the target of Tripterygii Wilfordii and the target of osteosarcoma. The yellow circle represents the target of Tripterygii Wilfordii, and the blue circle represents the target of the intersection of the 2 circles representing the target of Tripterygii wilfordii for osteosarcoma.

with OB > 30% and DL > 0.18 as the screening conditions to obtain the final active ingredients. In the Encyclopedia of Traditional Chinese Medicine (ETCM) database (http://www.tcmip.cn/ETCM/), we used as the keyword to obtain TW-related targets. The TCMSP database is a unique herbal system pharmacology platform that captures drug, target, and disease relationships. The database contains herbal compositions, oral bioavailability, drug likeness, intestinal epithelial penetration, blood-brain barrier, water solubility, and pharmacokinetic properties of chemical components of other natural compounds. The ETCM database integrates a large amount of standardized information on Chinese herbal medicines, formulations, and their ingredients, including herbal ingredients, herbs and formulations, as well as information on the taste, medicinal properties, attribution, and potential target genes of each herb, as well as the constructed network relationship map between Chinese herbal medicines and formulations, ingredients, target genes, related pathways and diseases.

2.2. Acquisition of OS-related targets

OS-related targets were obtained by searching the Disgenet (https://www.disgenet.org/) and Genecard databases (https://www.genecards.org/) using the keyword "OS." The targets were further integrated and duplicated to obtain the final OS-related therapeutic targets. The obtained targets were transformed to gene names using UniProt database (https://www.uniprot.org).

2.3. Constructing protein-protein interaction networks（PPI）

Venn plots were constructed for TW-related targets and OS-related targets. Genes at the intersection of the 2 circles were considered potential therapeutic targets for TW in treating OS. To explore the interactions between potential therapeutic targets, we imported the potential therapeutic targets into the STRING database (https://cn.string-db.org/) with a confidence level set to 0.9 and further analyzed and processed the obtained PPI network maps using Cytoscape software. The STRING database is a database for searching known protein-protein interactions and predicting protein-protein interactions, which can be applied to 2031 species and contains 9.6 million proteins and 13.8 million medium protein interactions. It includes experimental data, text mining from PubMed abstracts, and comprehensive data from other databases, in addition to results predicted using bioinformatic methods.

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

To investigate the potential molecular mechanisms in treating OS with TW, we performed GO enrichment analysis and KEGG

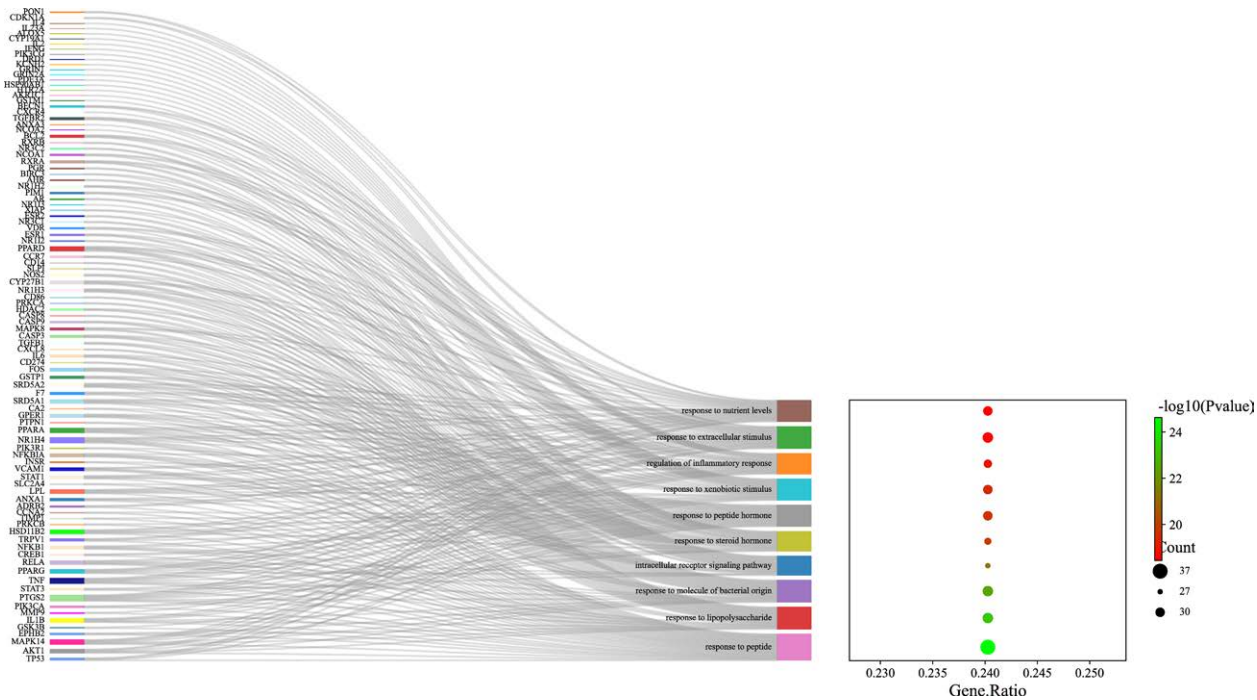

Figure 3. PPI network of Tripterygii Wilfordii in the treatment of osteosarcoma. The nodes represent potential therapeutic targets of Tripterygii wilfordii against osteosarcoma. The larger the node, the higher the corresponding target degree and the greater the number of connections to other nodes. PPI = protein-protein interaction networks.

Table 1

Characteristics of the hub gene.

Gene	Name	Degree	Betweenness centrality	Closeness centrality
RELA	Transcription factor p65	36	0.12339972	0.506072874
AKT1	RAC-alpha serine/threonine-protein kinase	31	0.114880055	0.486381323
PIK3R1	Phosphoinositide-3-kinase regulatory subunit alpha/beta/delta	30	0.06085849	0.469924812
STAT3	Signal transducer and activator of transcription 3	29	0.077673272	0.477099237
PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	27	0.055488101	0.46641791
TP53	Cellular tumor antigen p53	25	0.10262858	0.45620438
RXRA	Retinoic acid receptor RXR-alpha	25	0.100452903	0.43554007
TNF	Tumor necrosis factor	24	0.062614664	0.452898551
NR3C1	Glucocorticoid receptor	22	0.072331411	0.477099237
MAPK14	Mitogen-activated protein kinase 14	22	0.036514202	0.457875458
NFKB1	Nuclear factor NF-kappa-B p105 subuni	22	0.070883653	0.440140845
ESR1	Estrogen receptor	19	0.172943043	0.469924812
IL6	Interleukin-6	19	0.030907034	0.415282392
NCOA1	Nuclear receptor coactivator 1	19	0.065304617	0.396825397
NFKBIA	NF-kappa-B inhibitor alpha	17	0.025330975	0.437062937
STAT1	Signal transducer and activator of transcription 1-alpha/beta	16	0.01188063	0.416666667
FOS	Protein c-Fos	15	0.024374099	0.43554007
MAPK8	Mitogen-activated protein kinase 8	15	0.031606999	0.418060201
AR	Androgen receptor	14	0.01537103	0.413907285
CD40	Tumor necrosis factor receptor superfamily member 5	14	0.009943115	0.408496732
NCOA2	Nuclear receptor coactivator 2	14	0.016691696	0.384615385
PPARG	Peroxisome proliferator-activated receptor gamma	13	0.028590982	0.413907285
IL4	Interleukin-4	13	0.022988817	0.398089172
NOS2	Nitric oxide synthase, inducible	12	0.01328724	0.429553265
CDKN1A	Cip1-interacting zinc finger protein	12	0.012113576	0.403225806
MCL1	Induced myeloid leukemia cell differentiation protein Mcl-1	12	0.007167443	0.399361022
PPARA	Peroxisome proliferator-activated receptor alpha	12	0.009086226	0.39184953
RXRB	Retinoic acid receptor RXR-beta	12	0.013617936	0.378787879
BCL2	Apoptosis regulator Bcl-2	11	0.012506918	0.42662116
CASP8	Caspase-8	11	0.008019107	0.407166124
CXCL8	Interleukin-8	11	0.010171083	0.403225806
MT-CO2	Mitochondrially encoded cytochrome c oxidase ii	11	0.07783352	0.386996904
CASP3	Caspase-3	10	0.017665469	0.374251497
TGFB1	Transforming growth factor beta-1	9	0.017451489	0.39556962

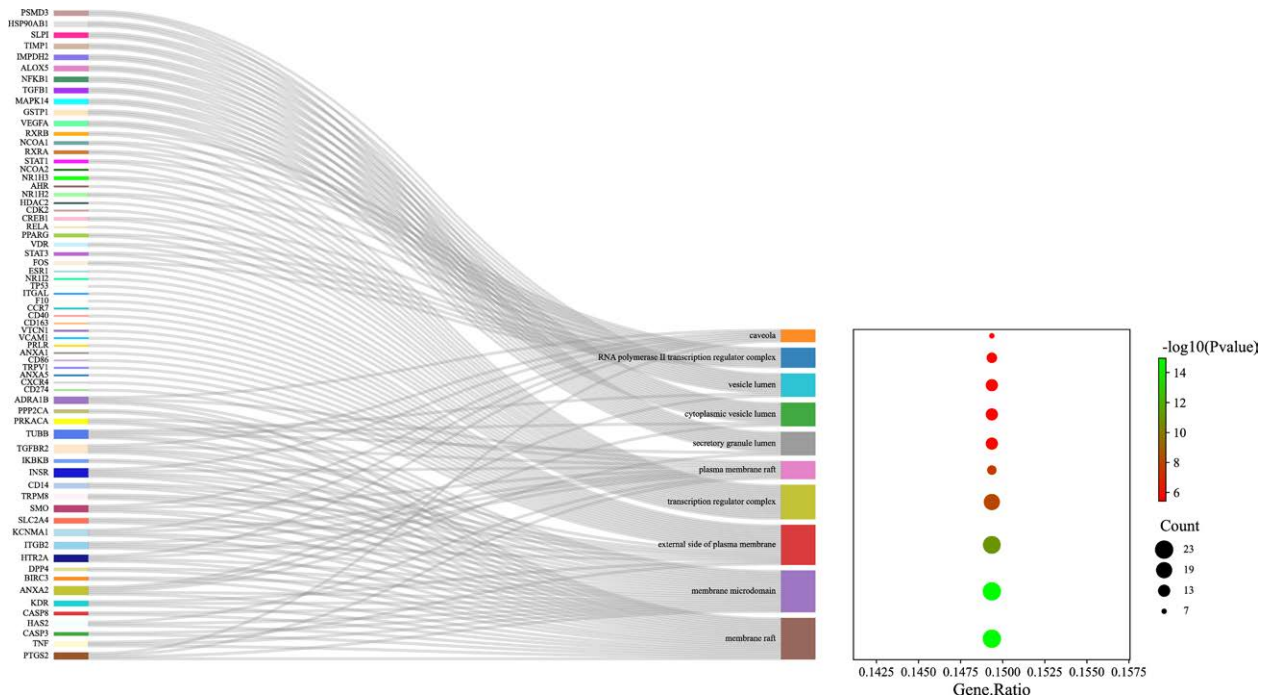

Figure 4. PPI network of the hub targets. The nodes represent hub targets. The larger the node, the higher the corresponding target degree and the greater the number of connections to other nodes. PPI = protein-protein interaction networks.

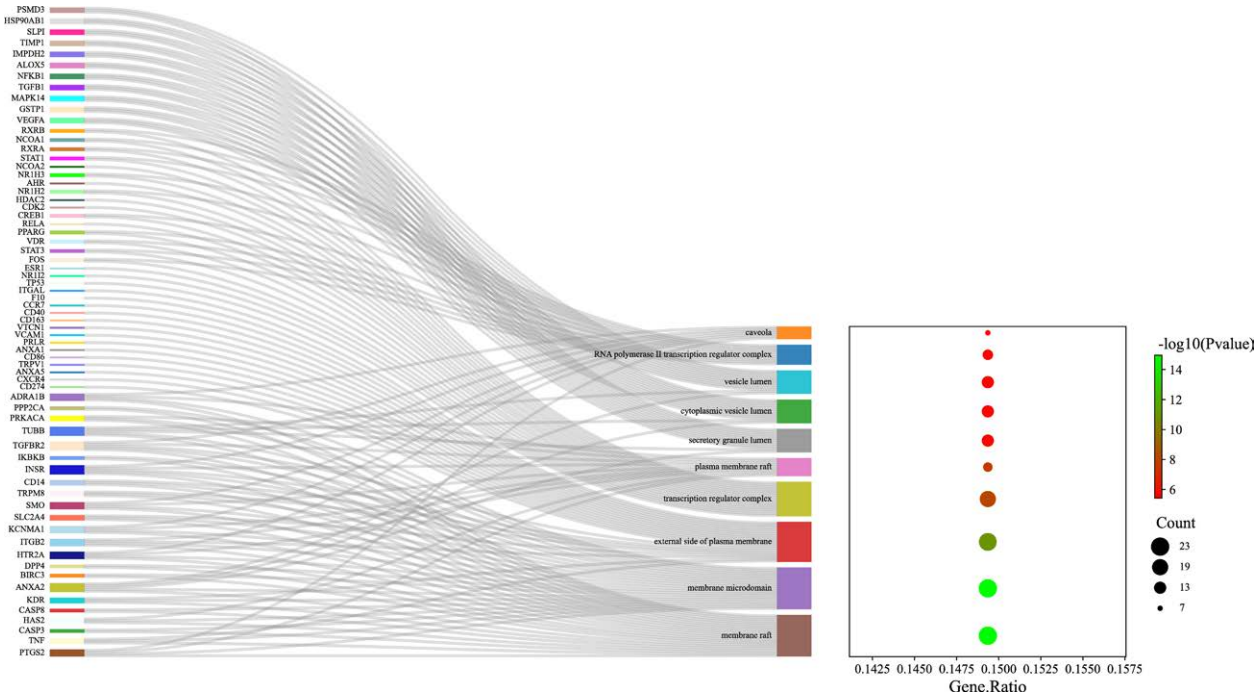

Figure 5. Top 10 significant biological process (BP) entries, and the relationship between the therapeutic targets and biological process.

enrichment analysis of potential therapeutic targets using the BiocManager package in R software (R x64 4.2.1). GO enrichment analysis was performed to explain expression in terms of biological processes (BP), cellular components (CC), and molecular functions (MF).

2.5. PPI construction, KEGG enrichment analysis of hub targets

Betweenness Centrality, Closeness Centrality, and Degree simultaneously greater than median potential target genes were used as hub genes. The medians of Betweenness Centrality, Closeness Centrality, and Degree were 0.017451489, 0.39556962, and 9, respectively. Hub genes were constructed and processed using the STRING database and Cytoscape software for the hub gene PPI network, and hub genes were analyzed by KEGG enrichment using the R package.

2.6. Plotting Kaplan–Meier (KM)curves

We investigated the effect of hub gene expression on the overall survival time in patients with OS. We obtained RNA sequence expression and clinical information of OS patients from The Cancer Genome Atlas Program database, screened and presented the RNA expression of each hub gene for each tissue, and plotted the KM curves of the hub genes using R software, with a significant KM curve of $P < .05$.

2.7. Molecular docking

To predict potential active ingredients for treating OS, hub targets that impact the overall survival time of OS patients were used for further molecular dock with the corresponding active ingredients of TW. The 3D structures of the target proteins were obtained from the PDB database (https://www.rcsb.org), and the 3D structures of the active ingredient small molecules were obtained from the TCMSP database. Autodock tools were used to preprocess the 3D structures of targets and small molecules, Autodock Vina was utilized for batch hub target docking, and Pymol software was used to visualize the docking results.

3. Results

3.1. Screening for potential active ingredients and targets of TW

We obtained 50 meaningful active ingredients through the TCMSP database screening (Supplementary Table 1, http://links.lww.com/MD/I184). A total of 139 TW targets were abained from TCMSP, and 334 potential therapeutic targets were obtained from the ETCM database. The TW-related targets acquired from the 2 databases were integrated and de-duplicated, and a total of 451 TW potential therapeutic targets were over-screened.

3.2. Screening for OS-related targets

4381 and 3763 OS-related targets were obtained from the Disgenet and Genecard database, respectively, and 4225 OS-related targets were obtained after integrating and de-duplicating the OS-related targets obtained from the 2 databases.

3.3. Construction of PPI network

We constructed Venn plots of TW-related targets and OS-related targets (Fig. 2), which were considered potential therapeutic target for treating OS in TW. The potential therapeutic targets were imported into the STRING database to construct a PPI network (Fig. 3), which was imported into Cytoscape software for further processing, analysis, and visualization. Based on the screening principle that potential target genes with betweenness centrality, closeness centrality, and degree greater than the median at the same time were used as hub genes, we obtained a total of 34 hub targets (Table 1). The hub genes were used in the same way to create the PPI network to explore the relationship between hub targets (Fig. 4).

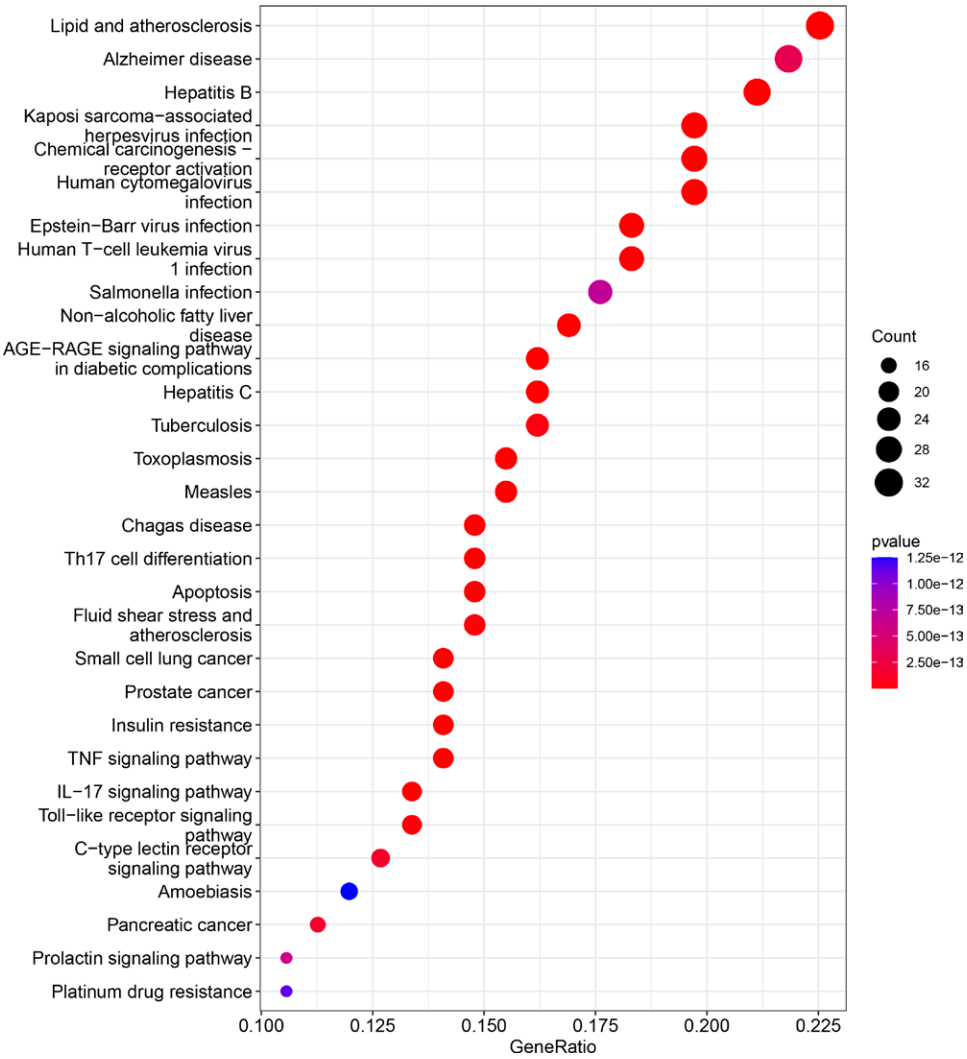

Figure 6. Top 10 significant cell component (CC) entries and the relationship between the therapeutic targets and cell component.

3.4. GO and KEGG enrichment analysis

To investigate the potential mechanism of thunderbolt in OS treatment, we performed GO and KEGG enrichment analyses of potential therapeutic targets using the R software BiocManager package. The top 10 GO enrichment results in terms of BP (Fig. 5), MF (Fig. 6), and CC (Fig. 7) highlight the relationship with the target genes; the KEGG enrichment results of the top 30 are shown (Fig. 8). We applied the same method to KEGG enrichment analysis of the hub genes (Fig. 9).

3.5. Plotting hub gene KM curves

To predict the effect of hub genes on the overall survival time of OS patients, we used the R package to process the genetic and clinical data of OS patients obtained from the Cancer Genome Atlas database (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) to plot KM curves, in which the differential expression of 3 genes, cellular tumor antigen p53 (TP53), Peroxisome proliferator-activated receptor gamma (PPARG), and signal transducer and activator of transcription 1-alpha/beta (STAT1), had a statistically significant effect on the overall survival time of patients ($P < .05$) (Fig. 10).

3.6. Molecular docking

To predict the binding of the active ingredient to the 3 target proteins mentioned above that can affect the overall survival time of patients with OS, we performed molecular docking of the hub target and the corresponding active ingredient, and the docking results were visualized using PyMOL software (Fig. 11 and Table 2). The free binding energy of the docking results < -4 Kcal/mol indicates that the target protein and the small molecule are freely bound. The free energy of docking results was plotted as a heat map using R software (Fig. 12).

4. Discussion

OS cells are derived from primitive mesenchymal cells.[4] It is most prevalent in adolescents and children.[3] The overall 5-year survival rate for patients with in situ OS is 60% to 70%,[19] and the overall 5-year survival rate for patients with recurrence or metastasis is 10% to 40%.[20] Although new treatments are constantly being attempted, the 5-year overall survival rate of patients has not changed for decades.[5] As OS predominates in adolescents and children,[1] distant metastases often occur at the time of diagnosis,[5] and the years of benefit from treatment for pediatric and adolescent OS patients are much lower than those for adults. There is an urgent need to discover new treatments or

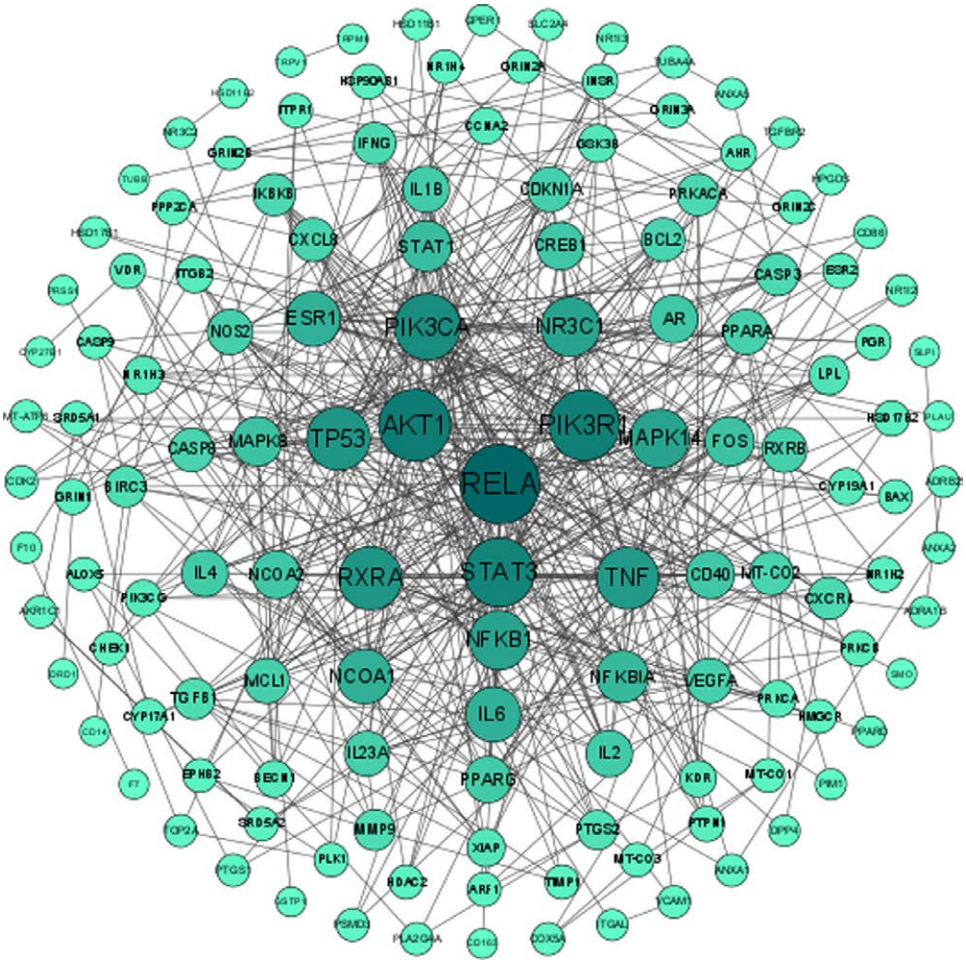

Figure 7. Top 10 significant molecular function (MF) entries, and the relationship between the therapeutic targets and molecular function.

therapeutic strategies to improve the overall survival of patients and the number of years of benefit for children and adolescents.

TW is a traditional Chinese herb that has been used for years to treat immune system disorders and has been extensively studied for its anti-inflammatory and immunomodulatory effects.[8] Recently, the antitumor effects of various active components of TW have been investigated.[21] Multiple active ingredients of TW, such as triptolide, celastrol, and tripchlorolide, have been shown to have anti-tumor effects.[9,15,22] However, it is unclear whether TW acts on OS and the exact mechanism of its action on OS.

This study used network pharmacology and molecular docking techniques to investigate whether TW acts on OS and its specific mechanism. Targets intersecting TW and OS were used as potential therapeutic targets. PPI network maps were constructed and analyzed for potential therapeutic targets, and GO and KEGG enrichment analyses were performed. The Hub genes obtained from the PPI analysis were used to construct KM curves using biological and clinical information from the TCGA database, and genes with $P < .05$ indicating that upregulation or downregulation of gene expression could prolong the overall survival time of OS patients.

The KEGG results suggest that the potential targets of TW in the treat OS are small cell lung cancer, prostate cancer, platinum resistance, interleukin-17 signaling pathway, TNF signaling pathway, apoptosis, C-type lectin receptor signaling pathway, and prolactin signaling pathway. The KEGG results showed that a hub target was often associated with multiple pathways, such as TP53 and small cell lung cancer, prostate cancer, apoptosis, platinum drug resistance, and pancreatic cancer, RELA was enriched in small cell lung cancer, interleukin-17 signaling pathway, TNF signaling pathway, apoptosis, C-type lectin receptor signaling pathway, and prolactin signaling pathway. The TNF signaling pathway has been widely reported to inhibit cell proliferation, induce apoptosis, and inhibit tumor cell migration and infiltration.[23,24] The interleukin-17 signaling pathway is an inflammatory pathway whose abnormal activation has been documented to promote the development of prostate and colon cancers and tumor growth.[25,26] Based on the PPI and KEGG enrichment results, we found that TW acts on each hub target and transmits signals along each signaling pathway by regulating the upregulation or downregulation of the hub target, forming a crosstalk signaling network through which the biological behavior of OS cells is affected. The KM curve results showed that the upregulated gene TP53, the downregulated gene PPARG, and STAT1 could prolong the overall survival time of patients, and the above 3 targets have the potential to become therapeutic targets for OS. The 3 were docked with the corresponding active small molecules, and the docking free energy <–4 kcal/mol indicated that the 2 could bind freely. The docking results indicated that all 3 targets could bind freely to the corresponding active small molecules. The top 3 free energies were triptolide (MOL003187) and TP53 docking (–7.2 kcal/mol), kaempferol (MOL000422) and PPARG docking (–7.2 kcal/mol), and 5,8-Dihydroxy-7-(4-hydroxy-5 methyl-coumarin-3)-coumarin (MOL003199) and PPARG docked (-7.2 kcal/mol). Triptolide, kaempferol, and 5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin have the potential to be used as drugs for OS treatment.

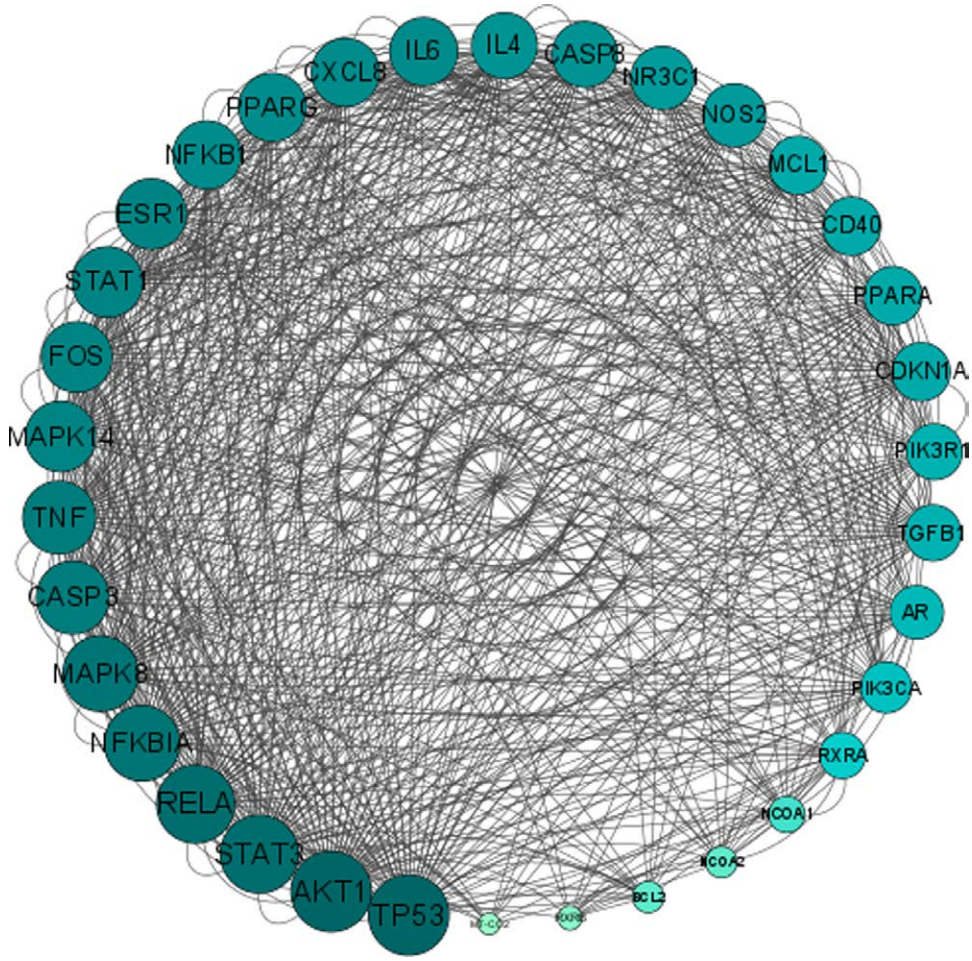

Figure 8. KEGG enrichment analysis for therapeutic targets. KEGG = Kyoto encyclopedia of genes and genomes.

Although network pharmacology has the advantages of low cost and high efficiency,[16–18] it is still a developing discipline and still has shortcomings, it can only be used for targets already discovered in the laboratory and cannot be used for the discovery of new targets; at the same time, this experiment still requires basic laboratory experiments for validation, In addition, because in this study, we used data from public databases, new targets may not be included in this study due to the existence of new databases not included.

5. Conclusion

Based on network pharmacology, this study demonstrates that TW affects the biological behavior of OS cells, including proliferation, apoptosis, migration, and infiltration. The treatment of OS with TW does not involve a single signaling pathway but multiple signaling pathways interconnected by hub targets to form a signaling network. Among the hub targets, upregulation of the TP53 gene and downregulation of PPARG and STAT1 gene can prolong the survival time of patients with OS, and the active ingredients acting on the these genes have the effect of becoming a treatment for OS and prolonging the survival time of patients. 5,8-Dihydroxy-7-(4-hydroxy-5 methyl-coumarin-3)-coumarin has the potential to become a drug for treating OS and prolong the survival time of OS patients.

Acknowledgments

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Author contributions

Y J designed the study. Yafang Zhang, Junqiang Wei, Mingze Song, Xiangyu Xiao and Yange Zhang conducted the study and analyzed the data. Yafang Zhang drafted the manuscript. Yu Jin revised the manuscript. (I) Conception and design: Y Jin; (II) Administrative support: Affiliated Hospital of Chengde Medical College; (III) Provision of study materials or patients: the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database, The Encyclopedia of Traditional Chinese Medicine (ETCM) database, the DisGeNET database, and the Genecard database; (IV) Collection and assembly of data: Y Zhang, J Wei, L Kong, H Cao, Y Zhang, X Xiao; (V) Data analysis and interpretation: Y Zhang, J Wei, L Kong, H Cao, Y Zhang, X Xiao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.
Conceptualization: Yafang Zhang.
Data curation: Yafang Zhang.
Formal analysis: Yafang Zhang, Yu Jin.
Funding acquisition: Yafang Zhang, Yu Jin.
Investigation: Yafang Zhang, Yu Jin.
Methodology: Yafang Zhang, Lingwei Kong, Mingze Song.

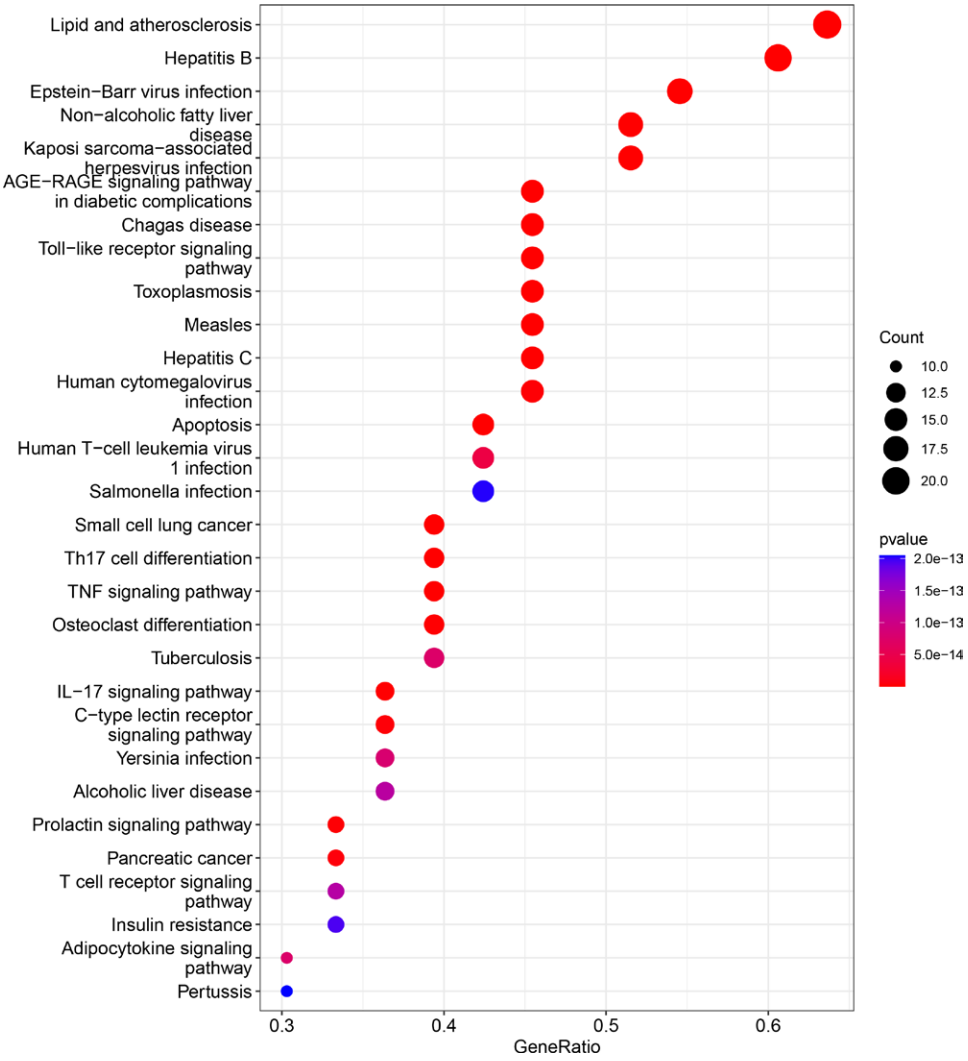

Figure 9. KEGG enrichment analysis for hub targets. KEGG = Kyoto encyclopedia of genes and genomes.

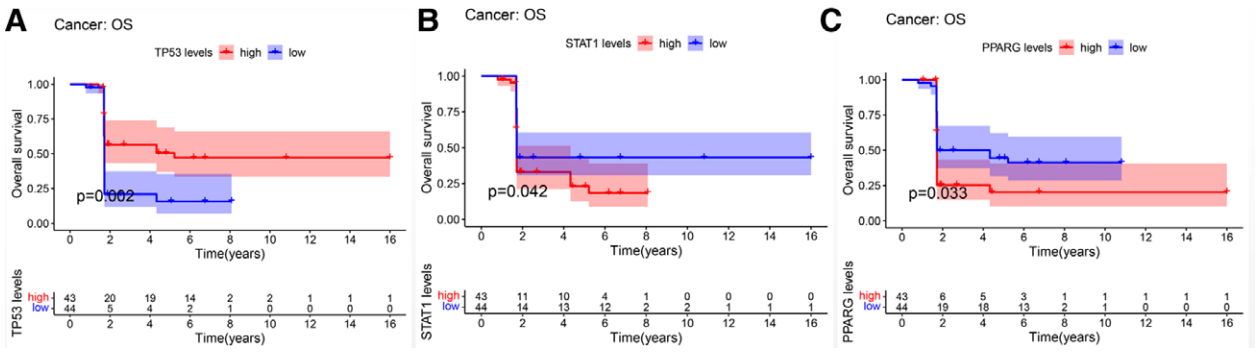

Figure 10. The Kaplan–Meier curves. A (TP53) is the KM curve for genes whose upregulation prolongs overall survival in patients with osteosarcoma. B (STAT1) and C (PPARG) are KM curves for genes whose downregulation could prolong the overall survival of patients with osteosarcoma. KM = Kaplan Meier.

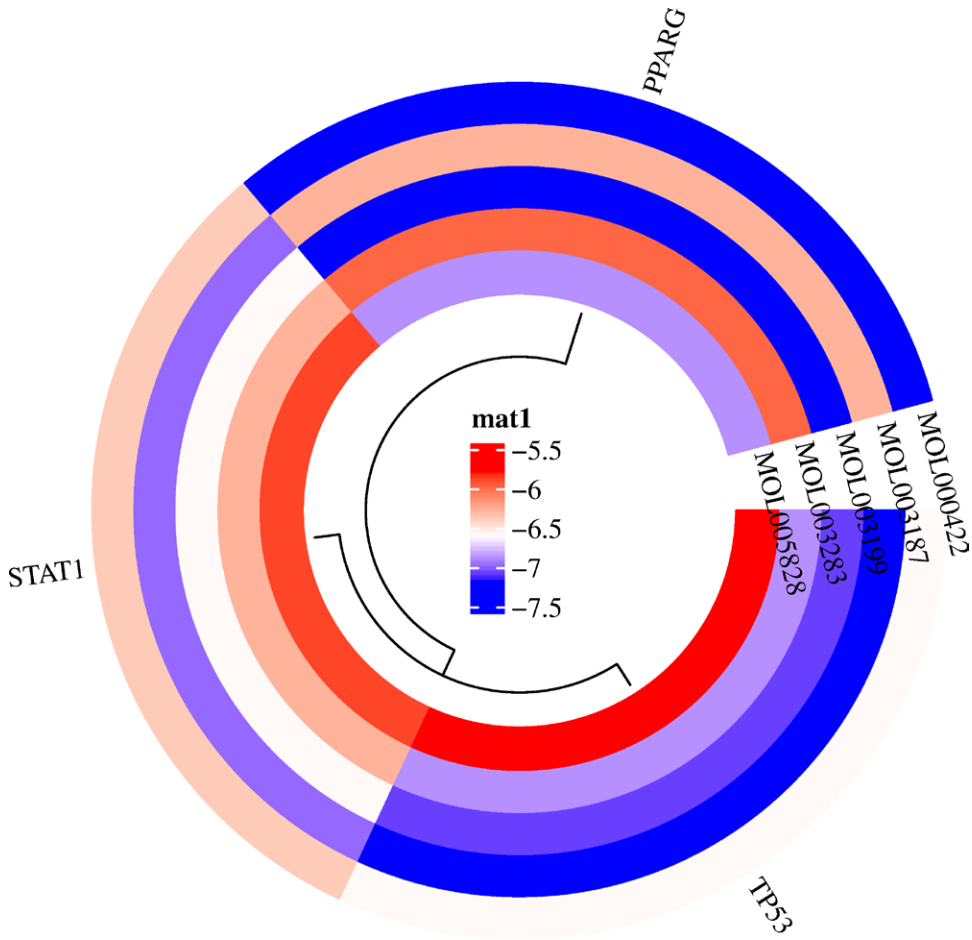

Figure 11. Heatmaps of the docking scores of hub genes combined with the bioactive compound of Tripterygii Wilfordii. The darker the blue, the more free energy the bioactive ingredient has to bind to the hub.

Table 2

Information on the docking results of the significant molecules.

Ligands	Receptor	Free energy (kcal/mol)	Corresponding serial numbers in Figure 11
Triptolide	TP53	−7.2	A
Nobiletin	TP53	−5.8	B
Kaempferol	PPARG	−7.2	C
5,8-Dihydroxy-7-(4-hydroxy-5-methylcoumarin-3-yl)coumarin	PPARG	−7.2	D
Isolariciresinol	PPARG	−6	E
Nobiletin	PPARG	−6.9	F
Triptolide	STAT1	−7	G

PPARG = peroxisome proliferator313 activated receptor gamma, STAT1 = signal transducer and activator of transcription 1-alpha/beta, TP53 = cellular tumor antigen p53.

Project administration: Yafang Zhang.
Resources: Yafang Zhang, Mingze Song.
Software: Yafang Zhang, Lingwei Kong, Mingze Song, Yange Zhang.
Supervision: Junqiang Wei, Yu Jin.
Validation: Yafang Zhang, Junqiang Wei, Haiying Cao.
Visualization: Yafang Zhang, Yange Zhang, Xiangyu Xiao, Haiying Cao.
Writing – original draft: Yafang Zhang.
Writing – review & editing: Yu Jin.

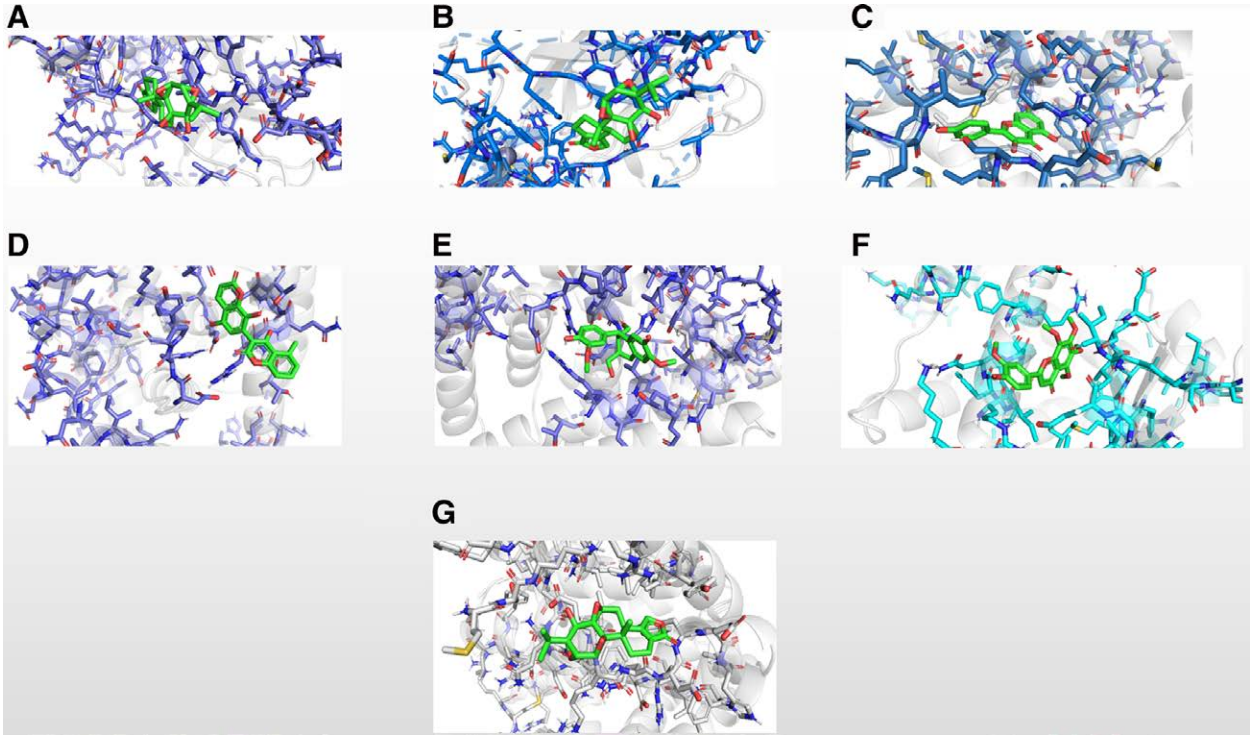

Figure 12. The significant Molecular Docking. A (Triptolide, TP53, -7.2kcal/mol), B(nobiletin, TP53, -5.8kcal/mol), C(kaempferol, PPARG, -7.2kcal/mol), D(5,8-Dihydroxy-7-(4-hydroxy-5-methylcoumarin-3-yl)coumarin, PPARG,-7.2kcal/mol), E(Isolariciresinol, PPARG, -6kcal/mol), F(nobiletin, PPARG,-6.9), G(Triptolide, STAT1, -7kcal/mol).

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