Network pharmacology and molecular docking reveal the mechanism of Angelica dahurica against Osteosarcoma

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Abstract
Osteosarcoma (OS) is a malignant bone tumor of mesenchymal origin. Angelica dahurica is a typical traditional Chinese herb. Angelica dahurica is used in the treatment of a variety of tumors. However, the studies of Angelica dahurica for OS have not been reported. To investigate Angelica dahurica’s potential mechanism of action in the treatment of OS, we used network pharmacology and molecular docking methods in this study. Of which the network pharmacology includes the collection of active ingredients of Angelica dahurica, the collection of predicted targets of Angelica dahurica and predicted targets of OS, the analysis of therapeutic targets of Angelica dahurica, gene ontology (GO) enrichment, and Kyoto encyclopedia of genes and genomes (KEGG) enrichment. The Venn plot performed showed that there were 225 predicted targets of Angelica dahurica for the treatment of OS. The therapeutic targets enrichment analysis results showed that Angelica dahurica treated OS through multiple targets and pathways. Angelica dahurica could affect OS's proliferation, apoptosis, migration, infiltration, and angiogenesis through a signaling network formed by pivotal genes crosstalking numerous signaling pathways. In addition, molecular docking results showed that senkyukangelicol, beta-sitosterol, and Prangenin, have a relatively high potential to become a treatment for patients with OS and improve 5-year survival in OS patients. We used network pharmacology and molecular docking methods to predict the active ingredients and significant targets of Angelica dahurica for the treatment of OS and, to a certain extent, elucidated the potential molecular mechanism of Angelica dahurica in the treatment of OS. This study provided a theoretical basis for Angelica dahurica in the treatment of OS.

Abbreviations: BP = biological process, CC = cellular component, ESR1 = estrogen receptor, ETCM = The Encyclopedia of Traditional Chinese Medicine, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, GO = gene ontology, IL1B = interleukin-1beta, IL6 = interleukin-6, INS = insulin, KEGG = Kyoto encyclopedia of genes and genomes, MF = molecular function, OS = osteosarcoma, PPARG = peroxisome proliferator-activated receptor gamma, KM = Kaplan–Meier, PPI = protein-protein interaction, PRKACA = cAMP-dependent protein kinase catalytic subunit alpha, RELA = transcription factor p65, RXRA = retinoic acid receptor RAR-alpha, TCMS = the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, TNF = tumor necrosis factor.

Keywords: Angelica dahurica, mechanism, molecular docking, network pharmacology, osteosarcoma

1. Introduction
Osteosarcoma (OS) is a primary malignant bone tumor of the skeletal system.[1] The annual morbidity rate of OS is 2 to 3 per million, with an incidence of 8 to 11 per million in adolescents in the 15 to 19 age group.[2] It often occurs in the epiphysis of the long stem bones of the body, such as the distal femur and the proximal tibia.[3] The current mainstream treatment for newly diagnosed OS patients is a combination of Pre-operative neoadjuvant chemotherapy, surgery, and postoperative adjuvant chemotherapy. About 30% to 40% OS patients have recurrence or distant recurrence within 2 to 3 years, and 90% of the distant metastases are in the lungs.[4] Patients with advanced OS tend to have a poor prognosis, with one study showing that the
5-year survival rate for patients with advanced OS is only 20% to 30%, compared to 65% to 70% for patients with early OS.\[4\] Based on traditional “sandwich” therapies, there have been tremendous advances in the treatment of OS, such as immunotherapy (immune checkpoint inhibitors) and targeted therapy (MYC-targeted drugs).\[5,6\] However, new treatment modalities are still of limited use due to the high side effects and small population size. Therefore, finding new therapeutic drugs and new therapeutic targets is crucial. Traditional Chinese medicine has been used in the anti-tumor field for a long time. Angelica dahurica is a typical traditional Chinese herb, and its active ingredients include coumarin (PubChem CID:323), phellopterin (PubChem CID:98608), imperatorin (PubChem CID:10212), oxyypeucedanin (PubChem CID:33306), byakangelicin (PubChem CID:10211), and pimpinellin (PubChem CID:4825), etc.\[7\] Many of them have been shown to exert anti-neoplastic effects by inhibiting tumor cell proliferation, promoting apoptosis, blocking the cell cycle, promoting autophagy, Suppressing the metastatic ability of tumor cells, and improving the sensitivity of drug-resistant tumor cells to chemotherapeutic drugs.\[8,9\] The literature has continuously reported that part of the active ingredients of Angelica dahurica can influence the development and progression of OS.\[10,11\] Due to herbal medicines’ diverse composition and functions, the specific mechanisms by which they exert their corresponding effects are challenging to be explored through basic experiments.\[12\] Network pharmacology is based on systems biology theory. It offers a new strategy for exploring the relationship between drugs and diseases by integrating systems biology, multidirectional pharmaceutical biology, bioinformatics, and computer science. Biosystems research has achieved a shift from the traditional single-drug, single-target model to a multi-drug, multi-target model through network pharmacology.\[13\] Network pharmacology allows for more efficiently building “component-protein/gene-disease” networks and revealing molecular regulatory mechanisms in a high-throughput manner.\[14\] These advantages of network pharmacology make it more convincing for the studies of multiple combination therapies. Therefore, this study intends to use network pharmacology and molecular docking to investigate the specific mechanism of Angelica dahurica in the treatment of OS.

2. Materials and Materials

The flow chart of the study design was shown in Figure 1.

2.1. Collection of active ingredients of Angelica dahurica

All the active ingredients of Angelica dahurica were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database (https://old.tcmsp.e.com/tcmsp.php), The active ingredients were retrieved and initially screened by adsorption, distribution, metabolism, and excretion with drug-like properties ≥0.18 and oral bioavailability ≥30, and the filtered data are used for further processing.

2.2. Collection of potential targets of Angelica dahurica

The action targets of Angelica dahurica were collected from the TCMSP database, The Encyclopedia of Traditional Chinese Medicine (ETCM) database (http://www.tc mip.cn/ETCM/), and symMap database (http://www.symmap.org/). TCMSP database: an independent online platform that provides essential information on Chinese herbal medicines, giving herbal ingredients, targets, and herbal-ingredient-target network diagrams. The ETCM database is a database providing information on targets, prescriptions, and related pathways of commonly used herbal medicines in traditional Chinese medicine. SymMap: an comprehensive database of traditional Chinese medicine enhanced by symptom mapping. In addition, the target proteins obtained from the TCMSP database need to be converted into gene IDs by the Uniprot database for the following data analysis. These databases obtained the screening species for the drug targets of Angelica dahurica for Homo sapiens. The obtained target genes were de-duplicated and then used in the following data processing step.

2.3. Collection of potential targets for OS

Human-associated OS genes were acquired from two databases. The DisGeNET database (https://www.disgenet.org/) and the Genecard database (https://www.genecards.org/). The Genecard database is a search platform that retrieves genes associated with human diseases from 150+ web sources. Genes were searched on the two platforms using the keyword “OS”. The detected data includes information about OS, such as the names and gene IDs of genes associated with OS. The genes retrieved in the DisGeNET database were initially screened for the obtained data using a score ≥0.8. Higher scores represent more confidence that the filtered data were associated with OS. The genes obtained from the above two platforms were integrated and de-duplicated, and the results were used for the next data analyses.

2.4. Construction of protein-protein interaction network

The screened Angelica dahurica-associated target genes and OS-associated genes were imported into the online Veen graph platform (https://bioinfogp.cnb.csic.es/tools/venny/) to make a venn graph about the relationship between the both of them and then screen out the overlapping genes. The sieved data were imported into the STRING platform (https://cn.string-db.org/) with the condition set to human and a confidence level of 0.7 to obtain the protein-protein interaction (PPI) network graph. The resulting PPI network was imported into Cytoscape software for further processing via the STRING app in Cytoscape software.

2.5. Hub gene analysis

The scores of each node of the imported PPI network diagram were analyzed and obtained through the network diagram analysis function in Cytoscape software (Cytoscape 3.8.0). The 20 hub genes with the highest scores in the treatment of OS with Angelica dahurica were identified for further data analysis.

2.6. Plotting Kaplan–Meier curves

The Kaplan–Meier Plotter online platform (https://kmplot.com/analysis/) is a platform that integrates data from Gene Expression Omnibus, The European Genome-phenome Archive, and the cancer genome atlas and analyzes patient survival times due to genetic differences. The hub genes obtained from Cytoscape software were plotted on Kaplan–Meier curves through the Kaplan–Meier Plotter online platform. Since there is no separate gene pool for OS, the object was sarcomas. The division between high and low groups was chosen as the median for gene expression levels.

2.7. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway analyses

To analyze the potential therapeutic targets and pathways of Angelica dahurica for OS. The target proteins of Angelica dahurica for OS were analyzed by GO enrichment analysis and KEGG enrichment analysis. The GO enrichment analysis was performed in terms of biological process (BP), cellular component (CC), and molecular function (MF). R-package-Bioconductor
Cluster Profiler is an R package (R x64 4.0.3) widely used for gene bioinformatics analysis.

2.8. Molecular docking stimulation

Autodock is the leading software for molecular docking to determine the plausibility of target genes. Its branch software Autodock 4 and Autodock vina were used in this study. In addition, Pymol software, a sub-software of python software, was also used to process and analyze the molecular docking results. Chem 3D software is typical for analyzing and processing chemical molecular structures. 2D structure of the active component was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and the 3D structure of the target protein was downloaded from PDB online platform (https://www.rcsb.org/). We imported the 2D structure of the

Figure 1. Network pharmacological study of Angelica dahurica for the treatment of osteosarcoma schematic diagram.
obtained active component into chem3D software to generate the 3D structure with minimal energy states. All the obtained 3D structures were imported into Autodock 4 software for dehyration and hydrogenation. The results were then subjected to bulk molecular docking using Autodock vina and Open Babel based on the principle of semi-flexible molecular docking. Energy \(<\ -7.0\ \text{kcal/mol}\) was used as a criterion for screening in the analyzed results. The top 20 most tightly connected docking results were filtered and imported into Pymol software for further processing, analysis, and generation images showing the bond and bond length between the molecule and the target protein.

2.9. Statistical analysis
For correlation analysis, spearman analysis was employed. The survival difference in different groups was compared using a log-rank test and illustrated in Kaplan–Meier (KM) survival plot. All statistical p values were two-sided, and in the paper, a p value \(<\ .05\) indicates statistical significance. Bioinformatics data analysis was performed with R x64 4.0.3, Cytoscape 3.8.0, the Kaplan–Meier Plotter online platform, and Microbiology mapping online tool.

3. Results

3.1. The chemical components of Angelica dahurica
22 active ingredients of Angelica dahurica were sieved from the TCMSP database by searching with the keyword Angelica dahurica and filtered with oral bioavailability \(\geq\ 30\) and drug-like \(\geq\ 0.18\). Table 1 is descriptive of the basic information of the 22 active ingredients.

3.2. Gene targets of Angelica dahurica
The genes were screened in the TCMSP database, ETCM database, and SymMap database with the keyword “Angelica dahurica,” The screened genes were integrated. The duplicates were removed to obtain 468 target genes of Angelica dahurica.

3.3. Gene targets of OS
The genes obtained from the two databases were further integrated and de-duplicated to get 4389 genes related to human OS, using “OS” as the keyword in the DisGeNET database and Genecard database.

3.4. Construction of PPI network
The 468 Angelica dahurica target genes and 4389 human OS-associated genes gained were used to make Veen plots (Fig. 2). Take the overlapping genes and import them into STRING online platform to make a PPI network map. The network diagram was imported into Cytoscape software for network diagram analysis and image processing to obtain Figure 3 and the basic information of the network diagram. And the 20 highest degree hub genes were screened (Table 2).

| Mol ID     | Molecule name             | MW (g/mol) | OB (%) | DL | CID         | MF: | Structure                        |
|------------|---------------------------|------------|--------|----|-------------|-----|----------------------------------|
| MOL001494  | Mandenol                  | 308.56     | 42     | 0.19 | 5282184     | C20H36O2 |
| MOL001939  | Alloisoimperatorin        | 270.3      | 34.8   | 0.22 | 5317436     | C16H14O4 |
| MOL001941  | Ammidin                   | 270.3      | 34.55  | 0.22 | 10212       | C16H14O4 |
| MOL001942  | Isoimperatorin            | 270.3      | 45.46  | 0.23 | 68081       | C16H14O4 |
| MOL001956  | Cnidilin                  | 300.33     | 32.69  | 0.28 | 821449      | C17H16O5 |
| MOL005789  | Neobyakangelicol         | 316.33     | 36.18  | 0.31 | 616064      | C17H16O5 |
| MOL005792  | (5-2'[R]-Hydroxy-3'-methyl-3'-butenyl-oxy)furocoumarin | 286.3 | 42.85 | 0.26 | 3009225 | C16H16O5 |
| MOL005800  | Byakangelicol            | 316.33     | 41.42  | 0.36 | 3055167     | C17H16O6 |
| MOL005802  | propyleneglycol monoleate | 340.61    | 37.6   | 0.26 | 5365625     | C2H4O3  |
| MOL005806  | 4-(2S)-2,3-dihydroxy-3-methylbutoxy)furo[3,2-g]chromen-7-one | 304.32 | 39.99 | 0.29 | 899657 | C2H16O6 |
| MOL005807  | Sen-byakangelicol        | 386.43     | 58     | 0.61 | 156995      | C2H2207  |
| MOL006358  | Beta-sitosterol           | 414.79     | 36.91  | 0.75 | 222284      | C21H500  |
| MOL006449  | Stigmastanol              | 412.77     | 43.83  | 0.76 | 5280794     | C21H480  |
| MOL007053  | Cholesterol (CLR)        | 386.73     | 37.87  | 0.68 | 5997        | C27H46O  |
| MOL008150  | Supraene                  | 410.8      | 33.55  | 0.42 | 638072      | C30H50  |
| MOL001749  | 2NC03860434               | 390.62     | 43.59  | 0.35 | 705792      | C4H3O4  |
| MOL002644  | Phellopterin              | 300.33     | 40.19  | 0.28 | 98608       | C17H16O5 |
| MOL003588  | Prangendrin               | 270.3      | 36.31  | 0.22 | 69502       | C16H14O4 |
| MOL003791  | Lindel, 2-mono-           | 354.59     | 37.28  | 0.3  | 5365676     | C2H16O5  |
| MOL007514  | Methyl Icosa-11,14-dienoate | 322.59  | 39.67  | 0.23 | 5365586     | C2H16O5 |
| MOL013430  | Prangnin                  | 286.3      | 43.6   | 0.29 | 182251      | C16H14O5 |

CID = pubchem ID, DL = drug-like properties, MF: = molecular formula, MW = molecular weight, OB = oral bioavailability.
Figure 3. PPI network of Angelica dahurica in the treatment of osteosarcoma. The nodes represent potential therapeutic targets of Angelica dahurica against osteosarcoma. The larger the node, the higher the corresponding target degree and the more connections to other nodes. PPI = protein-protein interaction.

Table 2
Characteristics of the top 20 hub gene.

| Gene  | Name                                                      | Degree | Betweenness centrality | Closeness centrality |
|-------|-----------------------------------------------------------|--------|------------------------|----------------------|
| AKT1  | RAC-alpha serine/threonine protein kinase                | 41     | 0.074511014            | 0.458333333          |
| JUN   | Transcription factor Jun                                  | 40     | 0.081636606            | 0.469849246          |
| MAPK3 | Mitogen-activated protein kinase 3                       | 38     | 0.088149814            | 0.469849246          |
| RELA  | Transcription factor p65                                 | 33     | 0.049678905            | 0.445238095          |
| INS   | Insulin                                                  | 31     | 0.07526763             | 0.43587436           |
| PIK3CA| Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform | 30     | 0.052243821           | 0.416481069          |
| TNF   | Tumor necrosis factor                                    | 29     | 0.017573751            | 0.418344519          |
| ESR1  | Estrogen receptor, ER                                     | 28     | 0.040724989            | 0.442083738          |
| IL6   | Interleukin-6                                            | 27     | 0.016264211            | 0.413716814          |
| PRKACA| cAMP-dependent protein kinase catalytic subunit alpha    | 26     | 0.161216456            | 0.424036281          |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase                 | 26     | 0.116940662            | 0.416481069          |
| RXRA  | Retinoic acid receptor RXR-alpha                          | 26     | 0.046459771            | 0.401287554          |
| CASP3 | Caspase-3                                                | 26     | 0.026587835            | 0.417410714          |
| IL1B  | Interleukin-1 beta                                       | 26     | 0.09440508             | 0.41067719           |
| NCOA1 | Nuclear receptor coactivator 1                           | 23     | 0.021056454            | 0.377016129          |
| CREB1 | Cyclic AMP-responsive element-binding protein 1          | 23     | 0.061751553            | 0.425968109          |
| PPARG | Peroxisome proliferator-activated receptor gamma         | 21     | 0.02776092             | 0.421171171          |
| PTEN  | Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN | 21     | 0.035509942           | 0.407407407          |
| PPARG | Peroxisome proliferator-activated receptor gamma         | 21     | 0.02776092             | 0.421171171          |
3.5. Plotting Kaplan–Meier curves

The 20 hub genes received were imported into the Kaplan–Meier Plotter online platform to plot the correlation curves. Ten genes in the results obtained were significant for survival time in sarcoma patients \( (p \text{ value} < .05) \). These ten genes are transcription factor p65 (RELA), Tumor necrosis factor (TNF), estrogen receptor (ESR1), interleukin-6 (IL6), cAMP-dependent protein kinase catalytic subunit alpha (PRKACA), interleukin-1 beta (IL1B), retinoic acid receptor RAR-alpha (RXRA), insulin (INS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Peroxisome proliferator-activated receptor gamma (PPARG). The Kaplan–Meier curves for these 10 genes are presented in Figure 4.

3.6. GO and KEGG enrichment analysis

To investigate the complex therapeutic mechanisms of the therapeutic targets of Angelica dahurica in patients with OS. We performed enrichment analysis in terms of BP, CC, and MF. The top 10 of BP, CC, and MF are shown in Figures 5A,B, 6A,B, and 7A,B. Figures 5B, 6B, and 7B highlight the relationship between target genes and BP, CC, MF. To further investigate the potential pathways of Angelica dahurica for the treatment of OS, Angelica dahurica targets for OS therapy were subjected to KEGG pathway enrichment analysis. And the top 30 results of the research are shown in Figure 8.

3.7. Molecular docking simulation

The 10 target genes with meaningful expression differences affecting the survival time of patients were docked to each of the 22 active components of Angelica dahurica. The docking results with energy \(< -7.0 \text{ kcal/mol} \) indicate that the receptor and ligand can bind to each other under natural conditions. The docking energy of 10 hub genes to 22 active molecules was used as a scoring criterion. All scores were plotted in Heatmaps (Fig. 9) to show the variability in the binding of hub genes to different components. The binding patterns of the top 20 tightest bindings in the docking results are shown in Figure 10. And the docking information for the top 20 is shown in Table 3.

4. Discussion

OS is a primary malignant bone tumor of mesenchymal origin with high metastatic and High malignancy features.\(^{[17]}\) Angelica dahurica is an herb commonly used in Chinese traditional medicine. According to the publications, its various Active components have been reported to have suppressive effects on OS.\(^{[12,13]}\) However, the specific OS-inhibiting effect of Angelica dahurica has not been reported.

In this study, several databases were availed to collect the active ingredients and targets related to Angelica dahurica and the targets associated with OS. The results of the veen diagrams of the two targets were obtained, and the overlapping part was taken as the potential target of Angelica dahurica for OS treatment. GO, KEGG enrichment analysis, and PPI network maps for the potential therapeutic targets obtained. The hub genes were obtained by analyzing the PPI network diagram, followed by making KM curves of the acquired hub genes, which impacted the survival time of sarcoma patients further dahurica active ingredients for molecular docking.

Based on the PPI network diagram, our obtained hub proteins, including RAC-alpha serine/threonine-protein kinase, EC 2.7.11.1 (AKT1), Transcription factor Jun (JUN), Mitogen-activated protein kinase 3 (MAPK3), RELA, INS, Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIK3CA), TNF, ESR1, IL6, PRKACA, GAPDH, RXRA, Caspase-3(CASP3), IL1B, Nuclear receptor coactivator 1(NCOA1), Cyclic AMP-responsive element-binding protein 1(CREB1), Peroxisome PPAR, Transcription factor Sp1(Sp1), Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN (PTEN) and peroxisome proliferator-activated receptor alpha
Using the KEGG results as a basis, we can see that these hub genes are not present in a stand-alone individual signaling pathway. For example, RELA is found on multiple pathways, such as the PI3K – Akt signaling pathway, MAPK signaling pathway, cAMP signaling pathway, HIF – 1 signaling pathway, TNF signaling pathway, MAPK signaling pathway, Toll – like receptor signaling pathway and C – type lectin receptor signaling pathway, etc. TNF is present in the TNF signaling pathway and found in the Toll – like receptor signaling pathway and C – type lectin receptor signaling pathway. These hub target genes interact by participating in multiple signaling pathways. This further suggests that these hub genes of Angelica dahurica are not through a single pathway on the biological behavior of OS. Still, through these hubs, crosstalk multiple pathways and interact with each other to form a signaling network, achieving a signal cascade amplification effect. In addition, The KEGG website shows that the above signaling pathways are divided into signaling-related pathways in Signal transduction of environmental information processing and immune system-related pathways. Pathways regarding Signal transduction of environmental information processing include the PI3K – Akt signaling pathway, MAPK signaling pathway, cAMP signaling pathway, HIF – 1 signaling pathway, TNF signaling pathway and C – type lectin receptor signaling pathway. A large number of previous studies have demonstrated that these signaling pathways affect various biological processes of OS, previous studies have shown that the MAPK signaling pathway, PI3K-Akt signaling pathway, and TNF signaling pathway affect the proliferation, apoptosis, migration,
and infiltration of OS cells. For example, DunxinHan et al. found that FGF5 promotes OS cell proliferation via activating the MAPK signaling pathway. Chao et al found that miR-652 regulates proliferation, apoptosis, migration, and infiltration of OS cells through the PI3K/Akt Signaling Pathway Yang et al. We further speculate that Angelica dahurica regulates the entire signaling network by modulating the role of hub proteins to control the proliferation, apoptosis, migration, and invasion of OS. 

Based on KM curve, we selected ten hub genes, RELA, TNF, ESR1, IL6, PRKACA, IL1B, RXRA, INS, GAPDH, PPARG. The median survival time of sarcoma patients is significantly prolonged when RELA, TNF, ESR1, IL6, PRKACA, IL1B, RXRA are upregulated, and INS, GAPDH, PPARG are downregulated. We hypothesize that the active ingredient acting on the hub mentioned above protein could influence the median survival time of OS patients as a potential active ingredient for treating OS. We performed a molecular docking simulation to investigate the relationship between the active molecule and the hub protein. Among the 220 docking results of docking, there are 61 binding modes capable of binding in the natural state (<−7.0 kcal/mol). The three active ingredients, sen-byakangelicol, beta-sitosterol, and Prangenin, docked the maximum number of successful target proteins. The three are likely

Figure 6. Top ten significant cell component (CC) entries. (A): GO enrichment analysis of therapeutic targets for cell component. (B): Relationship between the therapeutic targets and cell component. GO = gene ontology.
to be the main active ingredients of Angelica dahurica in the treatment of OS.

Network pharmacology is still a developing discipline. Although it has the advantages of low cost and high efficiency, it also has the disadvantage of not discovering unreported biological information. The discovery of new signaling factors still requires basic laboratory experiments to complete. But this study is not perfect. The limitations of this study include two aspects: firstly, the relevant targets in this study were obtained from some databases such as the TCMSP database, ETCM, and SymMap database. OS-related targets were extracted from the DisGeNET database and Genecard database. Each database has a different focus, and there are differences between databases; therefore, there may be potential risks in the joint analysis of multiple databases. Therefore, the discovery of new targets and pathways still needs to be done through basic laboratory experiments. Further basic experimental studies and clinical studies can be conducted to confirm the accuracy of this study. Secondly, when performing survival curve validation, the OS of the soft tissue sarcoma patients was validated, which provides a certain degree of feedback on the reliability of the network pharmacology analysis results, and more precise validation is needed for further development.

Figure 7. Top ten significant molecular function (MF) entries. (A): GO enrichment analysis of therapeutic targets for molecular function. (B): Relationship between the therapeutic targets and molecular function. GO = gene ontology.
5. Conclusion

Network pharmacology as a basis, this study demonstrates the potential role of Angelica dahurica in regulating biological processes such as proliferation, apoptosis, migration, and invasion of OS cells. And based on the results, it proves that the regulatory mechanism is not a single pathway but a signaling network. Each signaling pathway through the target protein crosstalk and influences each other, thus achieving the effect of treatment of OS. And ten core targets such as RELA, TNF, ESR1, IL6, PRKACA, IL1B, RXRA, INS, GAPDH, and PPARG...
were shown to have prolonged survival time in OS. Sen-byakangelicol, beta-sitosterol, and Prangenin are most likely the main components of Angelica dahurica in treating OS.

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Author contributions

Yu Jin designed this study. Yafang Zhang, Zhehong Li, Junqiang Wei, Mingze Song, Xiangyu Xiao, and Yange Zhang conducted this study and analyzed the data. Yafang Zhang and Zhehong Li drafted the manuscript. Yu Jin revised this study. (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, symMap database, the DisGeNET database, and the Genecard database; (IV) Collection and assembly of data: Y Zhang, Z Li, J Wei, L Kong, H Cao, Y Zhang, X Xiao; (V) Data analysis and interpretation: Y Zhang, Z Li, J Wei, L Kong, H Cao, Y Zhang, X Xiao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors

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Figure 10. The top twenty significant Molecular Docking. A (GAPDH, sen-byakangelicol, −9.6 kcal/mol); B (GAPDH, beta-sitosterol, −9.2); C (GAPDH, CLR, −8.6 kcal/mol); D (GAPDH, Prangenin, −8.4 kcal/mol); E (PPARG, sen-byakangelicol, −8.4); F (GAPDH, Cnidilin, −8.3 kcal/mol), G (GAPDH, Phenlopterin, −8.3 kcal/mol); H (GAPDH, neobyakangelicol, −8.2 kcal/mol); I (GAPDH, Pragenidin, −8.2 kcal/mol); J (PPARG, Stigmasterol, −8.2 kcal/mol); K (INS, Stigmasterol, −8.1 kcal/mol); L (GAPDH, Ammidin, −8.1 kcal/mol); M (GAPDH, furocoumarin, −8.1 kcal/mol); N (PPARG, CLR, −8); O (PPARG, isoimperatorin, −7.9 kcal/mol); P (GAPDH, isoimperatorin, −7.8 kcal/mol); Q (PPARG, Ammidin, −7.8 kcal/mol); R (INS; CLR; −7.7 kcal/mol); S (PPARG, Alloisoimperatorin, −7.7 kcal/mol); T (PPARG, chromen, −7.7 kcal/mol). INS = insulin, PPARG = peroxisome proliferator-activated receptor gamma.
### Table 3
Information on the docking results of the top 20 significant molecules.

| Ligands | Receptors | Free energy (kcal/mol) | Corresponding serial numbers in Figure 9 |
|---------|-----------|------------------------|------------------------------------------|
| GAPDH | Sen-byakangelicol | −9.6 | A |
| GAPDH | Beta-sitosterol | −9.2 | B |
| GAPDH | CLR | −8.6 | C |
| GAPDH | Prangenin | −8.4 | D |
| PPARG | Sen-byakangelicol | −8.4 | E |
| GAPDH | Cnidilin | −8.3 | F |
| GAPDH | Phellopterin | −8.3 | G |
| GAPDH | Neobyakangelicol | −8.2 | H |
| GAPDH | Prangenidin | −8.2 | I |
| PPARG | Stigmasterol | −8.2 | J |
| INS | Stigmasterol | −8.1 | K |
| GAPDH | Ammidin | −8.1 | L |
| GAPDH | Furocoumarin | −8.1 | M |
| PPARG | CLR | −8 | N |
| PPARG | Isoimperatorin | −7.9 | O |
| GAPDH | Isoimperatorin | −7.8 | P |
| PPARG | Ammidin | −7.8 | Q |
| INS | CLR | −7.7 | R |
| PPARG | Altoisoimperatorin | −7.7 | S |
| PPARG | Chromen | −7.7 | T |

GAPDH = glyceraldehyde-3-phosphate dehydrogenase, INS = insulin, PPARG = peroxisome proliferator-activated receptor gamma.

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