Original Article

Network pharmacology- and molecular docking-based investigation of the therapeutic potential and mechanism of daucosterol against multiple myeloma

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Background: Some studies have shown that daucosterol has potential anti-tumor activity, but its therapeutic effect on multiple myeloma (MM) has not been reported. This study aimed to evaluate the therapeutic effect daucosterol against MM and explore its possible mechanism through network pharmacology.

Methods: We collected daucosterol and approved drugs for MM, and their potential target profiles were obtained. We used 2 major methods to collect the gene sets related to the physiological process of MM. Based on the protein-protein interaction (PPI) network in the STRING database, the correlation between the therapeutic targets of daucosterol and MM-related genes was calculated by using the random walk with restart (RWR) algorithm to systematically evaluate the therapeutic potential of daucosterol for MM. On this basis, through intersection analysis, the potential targets of daucosterol in treating MM were identified, and the signaling pathways were mined. Furthermore, the key targets were identified. Finally, the regulatory relationship between the predicted daucosterol and potential targets was verified by molecular docking method, and the interaction mode between daucosterol and key targets was analyzed.

Results: A total of 13 approved drugs reported to treat MM were retrieved from the DrugBank database. A total of 35 potential targets of daucosterol were obtained, including 8 known targets and 27 newly predicted targets. In the PPI network, the target of daucosterol was significantly correlated with MM-related genes, indicating that it has therapeutic potential for MM. A total of 18 therapeutic targets for MM were obtained, which were significantly enriched in the FoxO signaling pathway, prostate cancer, the PI3K-Akt signaling pathway, insulin resistance, the AMPK signaling pathway, and pathways related to the regulation of *TP53*. The core targets were *HSP90AA1*, *MDM2*, *GSK3B*, *AKT3*, *PRKAA1*, and *PRKAB1*. Molecular docking suggested that daucosterol had potential direct regulatory effects on 13 of the 18 predicted targets.

Conclusions: This study highlights the use of daucosterol as a promising therapeutic drug for MM treatment. These data provide new insights into the potential mechanism of daucosterol in the treatment of MM, which may provide references for subsequent research and even the clinical treatment.

Keywords: Multiple myeloma (MM); daucosterol; network pharmacology; network targets; molecular docking

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Introduction

Multiple myeloma (MM) is a kind of malignant proliferative disease derived from monoclonal plasma cells. It is typically characterized by the production of a large amount of monoclonal immunoglobulin, which eventually leads to end-stage impairment of multiple organ function (1,2). MM is common among middle-aged and elderly patients, predominantly in elderly male patients (over 65 years old). The incidence of MM shows an increasing trend year by year (1,3), and currently ranks the second among hematological malignancies, second only to non-Hodgkin's lymphoma (4). In the past 30 years, high-dose chemotherapy, immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs), anti-CD38 monoclonal antibody, autologous hematopoietic stem cell transplantation, and chimeric antigen receptor T cells (CAR-T) have greatly improved the quality of life of patients, but MM currently remains incurable (5-7). During the treatment, patients may experience side effects such as diarrhea, thrombocytopenia, peripheral neuritis, pulmonary infection, and thrombosis (8,9). Clonal evolution of MM cells and an immunosuppressive tumor microenvironment are the main causes of relapse, progression, and drug resistance in MM (10,11). First-line drugs such as bortezomib are effective but expensive. Natural compounds with low side effects are cheap, easy to obtain, and have great potential application value in the treatment of various malignant tumors, including MM, and have attracted increasing attention (12,13).

Daucosterol is a natural small molecule compound. Relevant studies have reported its anti-tumor effect on liver cancer (14), breast cancer (15), prostate cancer (16), and non-small cell lung cancer (17). For example, it has obvious inhibitory effect on tumor cell proliferation, recurrence, and metastasis processes. The relevant mechanisms that have been studied include regulation of the JNK pathway (18), Wnt/β-catenin pathway (14), cell cycle (19), and oxidative stress (17). However, no previous study has investigated daucosterol in treatment of MM.

In recent years, network pharmacology has provided an efficient research strategy to elucidate the mechanism of action, discover new targets, and reposition new indications from the perspective of biological network balance (20-23). In this study, we preliminarily investigated the potential effect and mechanism of daucosterol against MM using network pharmacology and bioinformatics (*Figure 1*), laying the foundation for further exploration of its potential clinical application value. We present the following article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-456/rc).

Highlight box

Key findings

- In the PPI network, the target of daucosterol was significantly correlated with MM-related genes, indicating that it has therapeutic potential for MM.
- A total of 18 therapeutic targets for MM were obtained, which were significantly enriched in the FoxO signaling pathway, prostate cancer, the PI3K-Akt signaling pathway, insulin resistance, the AMPK signaling pathway, and pathways related to the regulation of *TP53*.
- The core targets were *HSP90AA1*, *MDM2*, *GSK3B*, *AKT3*, *PRKAA1*, and *PRKAB1*.

What is known and what is new?

- Some studies have shown that daucosterol has potential anti-tumor activity, but its therapeutic effect on MM has not been reported.
- This study highlights that daucosterol has promising therapeutic potential for MM treatment.

What is the implication, and what should change now?

- This study reflects the characteristics of pharmacological effects of natural products with "multiple targets and multiple pathways", which lays a foundation for the subsequent in-depth study of daucosterol in the treatment of MM and provides a basis for its application in the clinical treatment of MM.

Methods

Acquisition of potential target profiles of daucosterol and the approved drugs for MM

To systematically evaluate the therapeutic effect daucosterol against MM, we searched the DrugBank database (https://go.drugbank.com/) to identify and retrieve the approved small molecules drugs for the treatment of

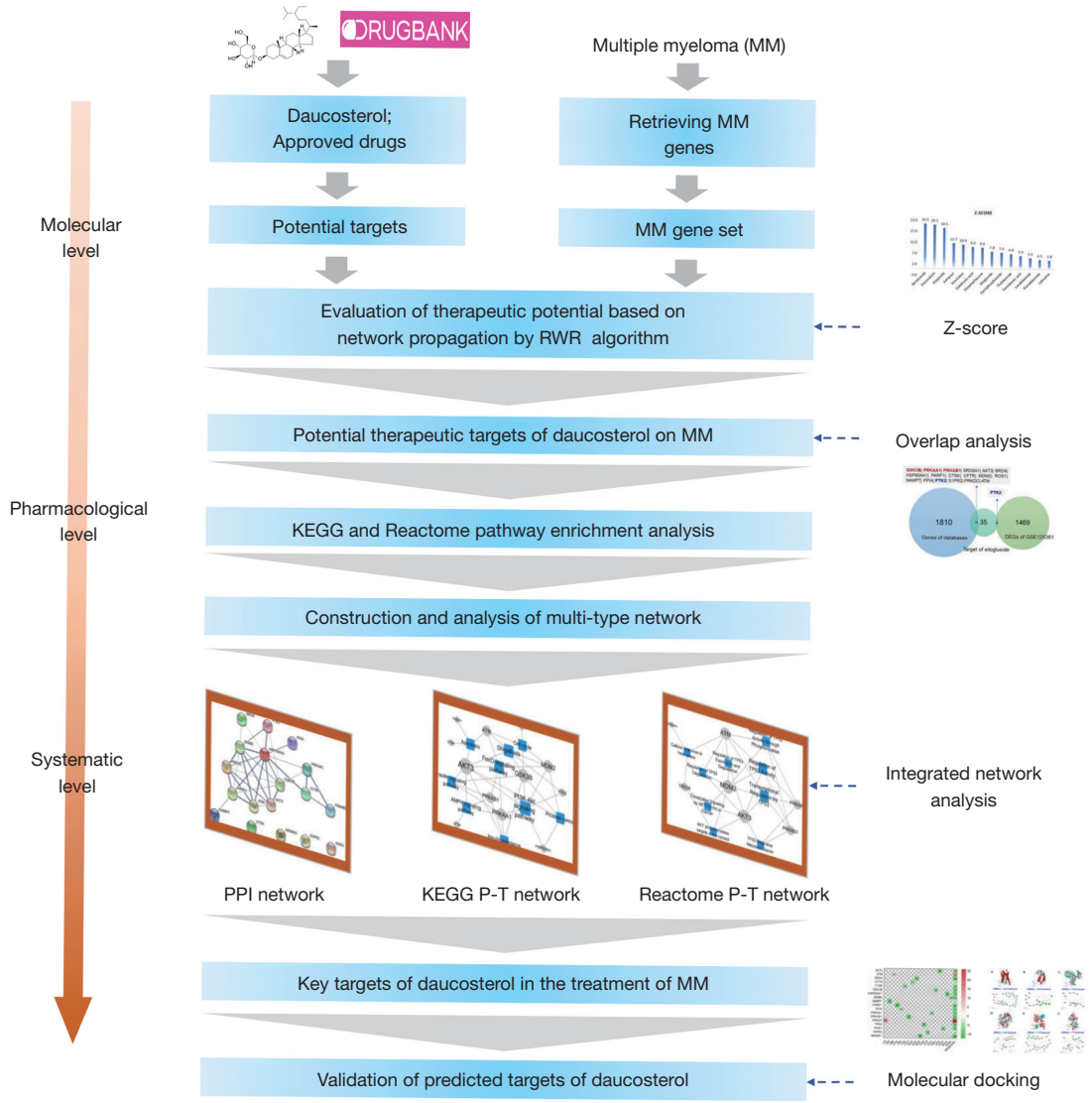

Figure 1 The detailed flowchart of network pharmacology and molecular docking to investigate therapeutic potential and mechanism of daucosterol against multiple myeloma. RWR, random walk with restart; KEGG, Kyoto Encyclopedia of Genes and Genomes; P-T, pathway-target; PPI, protein-protein interaction.

MM as reference. A comprehensive target spectrum of daucosterol and marketed drugs was constructed as the base to explore mechanism of action (24). To improve reliability, the known targets and the putative targets were integrated to obtain the target profiles of the drugs (*Table 1*). We defined targets from (I) marketed drug databases such as DrugBank (25) and Therapeutic Target Database (TTD) (26), (II) activity assay databases such as the Chemogenic European Molecular Biology Laboratory (ChEMBL) (27) and PubChem (28) as known targets. In addition, targets from (III) literature mining databases such as Search Tool for interactions of CHemicals and proteins (STITCH) (29), and (IV) target prediction tools such as Similarity Ensemble Approach (SEA) (30), TargetNet (31), SwissTargetPrediction (32), ChEMBL_prediction tool (33), and Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine (BATMAN-TCM) (34) were defined as putative targets. All the known targets were kept. Only the putative targets which could be predicted in at least 2 prediction models and validated by literature mining source at the same time were preserved. All of the targets were normalized by the UniProt database (http://

Table 1 List of databases used to obtain drug target profiles

Target source	Interaction type	Target classification	Reference
DrugBank	Known interaction	Known target	(25)
TTD	Known interaction	Known target	(26)
ChEMBL	Known interaction	Known target	(27)
PubChem	Known interaction	Known target	(28)
STITCH	Text mining interaction	Text mining target	(29)
SEA	Putative interaction	Putative target	(30)
TargetNet	Putative interaction	Putative target	(31)
SwissTargetPrediction	Putative interaction	Putative target	(32)
CHEMBL prediction	Putative interaction	Putative target	(33)
BATMAN-TCM	Putative interaction	Putative target	(34)

TTD, Therapeutic Target Database; ChEMBL, Chemogenic European Molecular Biology Laboratory; STITCH, Search Tool for interactions of CHemicals and proteins; SEA, Similarity Ensemble Approach; BATMAN-TCM, Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine.

www.uniprot.org) (35) and only those belonging to "*Homo sapiens*" were reserved for further analysis. In this study, we used the known target data as the validation data of the prediction target to verify the accuracy of the prediction method and ensure the reliability of the final compound target spectrum.

Construction of specific gene set of MM

We aimed to systematically construct specific molecular network highly related to the pathogenesis of MM. In order to achieve this goal, we integrated the published scattered scientific research results, which were collected from DisGeNET (36), Open Target Platform (37), MalaCards (38), Online Mendelian Inheritance in Man (OMIM) (39), GeneCards (40), and the Comparative Toxicogenomics Database (CTD) (41). To ensure the credibility of MM gene set, we only retained genes that appeared in at least 4 data sources.

In addition, we integrated differentially expressed genes (DEGs) analyzed from GSE125361 by the GEO2R tool in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) (screening threshold: $P<0.05$ and $|FC| \geq 1.2$) (42). The gene symbol was standardized based on the Database for Annotation, Visualization, and Integrated Discovery (DAVID) knowledgebase (v2022q2) (https://david.abcc.ncifcrf.gov/) (43).

Evaluation of drug efficacy in view of network propagation

In order to systematically and comprehensively evaluate the efficacy of a daucosterol and marketed drug to MM, we computed the correlation of the drug's targets and the genes associated with the disease in view of network propagation. First, we took drug targets and disease genes as seed nodes respectively to run random walk with restart (RWR) algorithm (44) in the background network obtained from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database version 11.5 (https://cn.string-db.org/) (45). In this way, we obtained the influence score vector of the 2 seed sets against all nodes in the background network, respectively. Further, the Pearson correlation coefficient of the 2 score vectors (*Cor*) was calculated and the significance was evaluated the by Z-score. The higher the value, the stronger the correlation between the drug and disease, and significance was generally considered if the value was greater than 3:

$$Z\text{-}score = \frac{Cor - E(Cor)}{\delta(Cor)} \quad [1]$$

where $E(Cor)$ and $\delta(Cor)$ were mean and standard deviation of the Pearson correlation coefficients between influence score vector of drug targets and those of 1,000 groups of random contrast disease genes, each of which contained the same number of randomly selected proteins as the disease seed nodes. The RWR algorithm was performed in R 3.5.2

by package dnet (version 1.1.4; R Foundation fr Statistical Computing, Vienna, Austria), and the restart probability was set as the default value 0.75.

Identification of potential therapeutic targets of daucosterol against MM

The targets of daucosterol that overlapped with genes of MM were selected as the candidate targets with therapeutic effects on MM.

Enrichment analysis

For the sake of interpreting the mechanisms of daucosterol against MM from a systematic perspective, clusterProfiler Version 4.0.3, an R Bioconductor package (46) was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome pathway enrichment analysis (47).

Networks construction

Based on the relationship between the potential target of daucosterol and the gene sets related to MM, we constructed a component-target-disease network to demonstrate the potential mechanism of action of daucosterol in the treatment of MM. Cytoscape v3.7.1 was used to visualized the network (48). To further identify the key targets of daucosterol in the regulation of MM, we constructed a protein-protein interaction (PPI) network through STRING (45). It contains information on more than 14,000 species, more than 60 million proteins, and more than 20 billion interactions. These protein interactions include both direct physical interactions and indirect functional correlations.

To verify that the interactions between them were statistically valid, PPI interaction enrichment tests were conducted. Whole genome was assumed as the statistical background. The hub nodes in the networks were evaluated by the crucial topological parameters, namely degree (49). The NetworkAnalyzer plugin (50) of Cytoscape was used to compute the degree. The degree of a node was the combined number of neighbor nodes (49).

Verification of interaction by molecular docking

The molecular docking method was used to predict the binding affinity between daucosterol and the potential therapeutic targets for the treatment of MM, which could verify the predicted interaction relationship at the molecular level and provide a basis for further experimental verification. AutoDock Vina 1.1.2 (https://vina.scripps.edu/) was used to conduct the docking task (51). The spatial data files (SDFs) of compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (28) and converted to MOL2 format by Open Babel 2.4.1 (52). We obtained 3-dimensional (3D) structures of the proteins from the Protein Data Bank (PDB) database (https://www. rcsb.org/) (53). The ligands and receptors were prepared according to the tutorial of AutoDock Vina 1.1.2. For each structure, we deleted the water molecules, added non-polar hydrogen, calculated the Gasteiger charge, and saved them as Protein Data Bank, partial charge (Q) and Atom Type (T) (PDBQT) format. The lower the binding energy, the more stable the ligand-receptor binding conformation (threshold: ≤–5.0 kJ/mol). The conformation with the lowest affinity was used as the best docking conformation and protein-ligand interaction profiler (PLIP) was taken to visualize the interaction mode (54).

Results

Target profile of daucosterol and the approved drugs for MM

A total of 13 approved drugs reported to treat MM were retrieved from the DrugBank database (*Table 2*). Multiple types of target data sources were used to obtain the target spectra of daucosterol and the approved drugs. The detailed targets, data sources, and target types are listed in https://cdn.amegroups.cn/static/public/tcr-23-456-1.xlsx, and the number of potential targets corresponding to each drug is listed in *Table 3*.

Gene set of MM

To make the study more rigorous, we obtained MM-related gene sets in 2 ways. The first was database retrieval. By searching multiple databases and integrating genes from at least 4 data sources, 1,810 pathogenic genes of MM were finally obtained (available online: https://cdn.amegroups.cn/static/public/tcr-23-456-2.xlsx). The second was by analyzing the omics data of MM (GSE125361). We obtained 1,469 DEGs, including 336 down-regulated and 1,133 down-regulated genes (screening threshold: $P<0.05$ and $|FC| \geq 1.2$) (available online: https://cdn.amegroups.cn/static/public/tcr-23-456-3.xlsx). The gene sets obtained by the above

Table 2 Basic information of daucosterol and marketed drugs for the treatment of multiple myeloma

#	Compound	CID	CHEMBL ID	Molecular formula	Molecular weight (g/mol)
1	Cyclophosphamide	2907	CHEMBL88	C7H15Cl2N2O2P	261.08
2	Lidocaine	3676	CHEMBL79	C14H22N2O	234.34
3	Pamidronic acid	4674	CHEMBL834	C3H11NO7P2	235.07
4	Thalidomide	5426	CHEMBL468	C13H10N2O4	258.23
5	Dexamethasone	5743	CHEMBL384467	C22H29FO5	392.5
6	Vincristine	5978	CHEMBL90555	C46H56N4O10	825
7	Doxorubicin	31703	CHEMBL53463	C27H29NO11	543.5
8	Etoposide	36462	CHEMBL44657	C29H32O13	588.6
9	Zoledronic acid	68740	CHEMBL924	C5H10N2O7P2	272.09
10	Pomalidomide	134780	CHEMBL43452	C13H11N3O4	273.24
11	Lenalidomide	216326	CHEMBL848	C13H13N3O3	259.26
12	Bortezomib	387447	CHEMBL325041	C19H25BN4O4	384.2
13	Daucosterol	5742590	CHEMBL506678	C35H60O6	576.8
14	Selinexor	71481097	CHEMBL3545185	C17H11F6N7O	443.3

CID, Pubchem_ID.

Table 3 Statistics on the types and number of targets of daucosterol and marketed drugs in treating multiple myeloma

#	Compound	CID	Known target	Putative target	Known & putative target	Total
1	Cyclophosphamide	2907	10	44	0	54
2	Lidocaine	3676	13	173	1	187
3	Pamidronic acid	4674	3	47	2	52
4	Thalidomide	5426	16	87	3	106
5	Dexamethasone	5743	20	74	6	100
6	Vincristine	5978	34	26	8	68
7	Doxorubicin	31703	55	20	11	86
8	Etoposide	36462	51	16	7	74
9	Zoledronic acid	68740	8	29	8	45
10	Pomalidomide	134780	2	74	4	80
11	Lenalidomide	216326	7	69	2	78
12	Bortezomib	387447	124	13	6	143
13	Daucosterol	5742590	8	27	0	35
14	Selinexor	71481097	4	23	0	27

CID, Pubchem_ID.

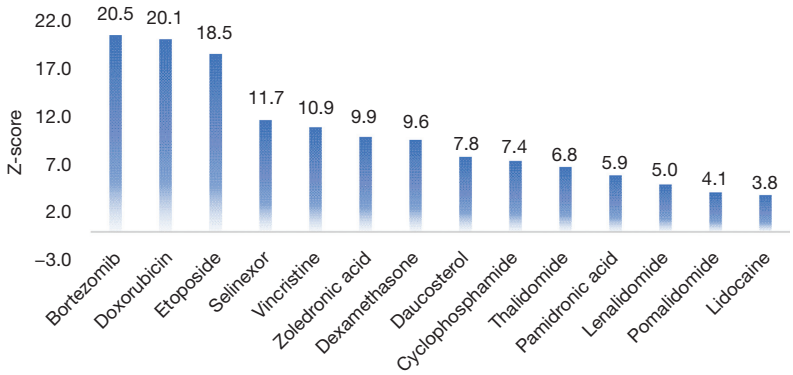

Figure 2 Network-based evaluation of the therapeutic potential of drugs for multiple myeloma. The higher the value, the stronger the correlation between the drug and disease, and significance is generally considered if the value is greater than 3.

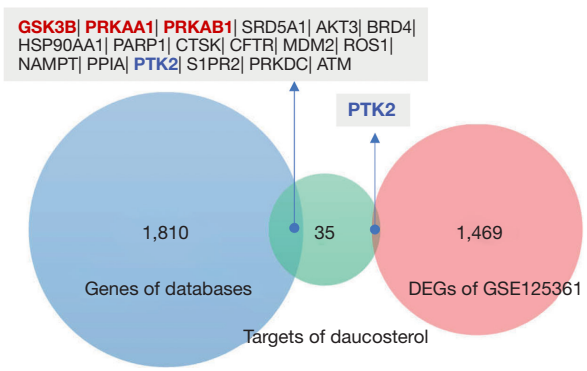

Figure 3 Potential therapeutic targets of daucosterol in the treatment of multiple myeloma. Targets in bold red are known targets. Target in bold blue is a common gene of multiple myeloma derived from databases and GSE125361. DEG, differentially expressed gene.

2 methods were used for subsequent research on the mechanism and targets of daucosterol in the treatment of MM.

Daucosterol showed the potential in the treatment of MM

In this study, we used a network-based approach to comprehensively evaluate the therapeutic potential of daucosterol for MM, using marketed drugs as controls (*Figure 2*). The results showed that targets profiles of daucosterol and MM-related genes were significantly correlated in the PPI background network, suggesting that daucosterol has the potential to treat MM.

Potential therapeutic targets of daucosterol in the treatment of MM

By intersection analysis of daucosterol target profiles with MM-related gene sets from 2 sources, a total of 18 potential targets were obtained (*Figure 3*), including 3 known targets (GSK3B, PRKAA1, PRKAB1) and 15 novel predicted targets.

Core signaling pathways potentially regulated by daucosterol in the treatment of MM

To interpret the mechanisms of daucosterol against MM from a systematic perspective, KEGG and Reactome pathway enrichment analysis were conducted (*Table 4*). The top 5 significantly enriched KEGG pathways were the FoxO signaling pathway (P.adjust=0.0005), prostate cancer (P.adjust=0.0017), PI3K-Akt signaling pathway (P.adjust=0.0005), insulin resistance (P.adjust=0.0017), and the AMPK signaling pathway (P.adjust=0.0017). The top 5 significantly enriched Reactome pathways were AKT phosphorylates targets in the cytosol (P.adjust=0.0003), regulation of TP53 activity (P.adjust=0.0004), constitutive signaling by AKT1 E17K in cancer (P.adjust=0.0006), regulation of TP53 activity through phosphorylation (P.adjust=0.0006), and regulation of TP53 degradation (P.adjust=0.001).

Identification of key targets of daucosterol in the treatment of MM

A PPI network of 18 potential functional targets of

Table 4 Top 9 enriched KEGG and Reactome pathways respectively

ID	Pathway name	P.adjust	Gene symbol
hsa04068	FoxO signaling pathway	0.0005	*PRKAA1/PRKAB1/AKT3/MDM2/ATM*
hsa05215	Prostate cancer	0.0017	*GSK3B/AKT3/HSP90AA1/MDM2*
hsa04151	PI3K-Akt signaling pathway	0.0017	*GSK3B/PRKAA1/AKT3/HSP90AA1/MDM2/PTK2*
hsa04931	Insulin resistance	0.0017	*GSK3B/PRKAA1/PRKAB1/AKT3*
hsa04152	AMPK signaling pathway	0.0018	*PRKAA1/PRKAB1/AKT3/CFTR*
hsa05131	Shigellosis	0.0018	*GSK3B/AKT3/MDM2/PTK2/ATM*
hsa04110	Cell cycle	0.0018	*GSK3B/MDM2/PRKDC/ATM*
hsa04210	Apoptosis	0.0018	*AKT3/PARP1/CTSK/ATM*
hsa04910	Insulin signaling pathway	0.0018	*GSK3B/PRKAA1/PRKAB1/AKT3*
R-HSA-198323	AKT phosphorylates targets in the cytosol	0.0003	*GSK3B/AKT3/MDM2*
R-HSA-5633007	Regulation of TP53 Activity	0.0004	*PRKAA1/PRKAB1/AKT3/MDM2/ATM*
R-HSA-5674400	Constitutive signaling by AKT1 E17K in cancer	0.0006	*GSK3B/AKT3/MDM2*
R-HSA-6804756	Regulation of TP53 activity through phosphorylation	0.0006	*PRKAA1/PRKAB1/MDM2/ATM*
R-HSA-6804757	Regulation of TP53 degradation	0.0010	*AKT3/MDM2/ATM*
R-HSA-6806003	Regulation of TP53 expression and degradation	0.0010	*AKT3/MDM2/ATM*
R-HSA-3700989	Transcriptional regulation by TP53	0.0059	*PRKAA1/PRKAB1/AKT3/MDM2/ATM*
R-HSA-5628897	TP53 regulates metabolic genes	0.0086	*PRKAA1/PRKAB1/AKT3*
R-HSA-3371556	Cellular response to heat stress	0.0086	*GSK3B/HSP90AA1/ATM*

KEGG, Kyoto Encyclopedia of Genes and Genomes.

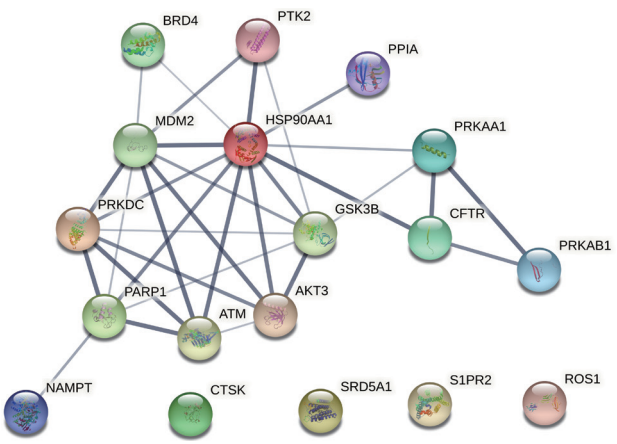

Figure 4 PPI network of 18 anti-multiple myeloma functional targets of daucosterol. PPI, protein-protein interaction.

daucosterol for MM treatment was constructed based on the STRING database (*Figure 4*), and the network parameters are listed in *Table 5*. The PPI network consisted of 18 nodes and 32 edges. The average node degree and the average local clustering coefficient were 3.56 and 0.606, respectively. PPI interaction enrichment result indicated that this PPI sub-network has significantly more interactions than what would be expected for a random set of proteins of similar size, drawn from the genome (P=1.07e-07). This means that these proteins are closely connected biologically as a group. The degree of nodes in the network was calculated (*Table 6*).

In order to accurately locate the core targets of daucosterol in the treatment of MM, we integrated the results of pathway enrichment analysis and selected the top 5 signaling pathways to construct pathway-target network

Table 5 PPI network statistical parameters

Network statistical parameters	Value
Number of nodes	18
Number of edges	32
Average node degree	3.56
Avg. local clustering coefficient	0.606
Expected number of edges	11
PPI enrichment P value	1.07e-07

PPI, protein-protein interaction; Avg., average.

Table 6 Node degree of target in PPI network and P-T network

Gene symbol	Degree in PPI network	Degree in KEGG P-T network	Degree in Reactome P-T network
HSP90AA1	11	2	1
MDM2	8	5	7
*GSK3B**	7	6	3
PARP1	6	1	0
PRKDC	6	1	0
AKT3	5	8	7
ATM	5	4	6
*PRKAA1**	4	5	4
CFTR	3	1	0
PTK2	3	2	0
BRD4	2	0	0
*PRKAB1**	2	4	4
NAMPT	1	0	0
PPIA	1	0	0
CTSK	0	1	0
ROS1	0	0	0
S1PR2	0	0	0
SRD5A1	0	0	0

*, the known targets. PPI, protein-protein interaction; P-T, pathway-target; KEGG, Kyoto Encyclopedia of Genes and Genomes.

(P-T network) (*Figure 5*). It is generally believed that targets involved in multiple signaling pathways play an important role in cross-talk between different signaling pathways.

By integrating PPI network and P-T network data, it is very easy to select the core targets of daucosterol in the treatment of MM, which occupy an important network topological position in the PPI network and play the role of cross-talk in multiple signaling pathways, including *HSP90AA1*, *MDM2*, *GSK3B*, *AKT3*, *PRKAA1*, and *PRKAB1* (*Table 6*).

Daucosterol might have potential direct regulatory effects on target proteins closely related to MM

Molecular docking was conducted to verify the predicted interaction relationship between daucosterol and the potential therapeutic targets. If the binding free energy is lower than the threshold (≤–5.0 kJ/mol), it indicates that the compound has the potential of forming stable binding conformation with the target protein (*Figure 6*). The binding free energy between daucosterol and 13 of the 18 targets was lower than the threshold, indicating that they could easily form stable binding conformations. Among them, the binding free energy of 6 targets with daucosterol was lower than that with co-crystallized molecules, including *BRD4* (affinity =–10 kcal/mol), *GSK3B* (affinity =–7.9 kcal/mol), *GSK3B* (affinity =–7.9 kcal/mol), *CTSK* (affinity =–7.4 kcal/mol), *PRKAA1* (affinity =–7.3 kcal/mol), *MDM2* (affinity =–6.5 kcal/mol), and PPIA (affinity =–6.3 kcal/mol). These results demonstrated the reliability of daucosterol's potential target profile and its therapeutic potential with MM.

Interaction patterns between daucosterol and the 6 targets with the lowest binding free energy were visualized (*Figure 7*). The interaction modes with the other 7 targets can be seen in Figure S1. The results showed that daucosterol could successfully dock with the active pocket of the core targets in the treatment of MM, revealing a stable docking model with a specific binding site, binding distance, and binding atom. For example, the *SRD5A1*-daucosterol complex was stabilized at amino acid residue

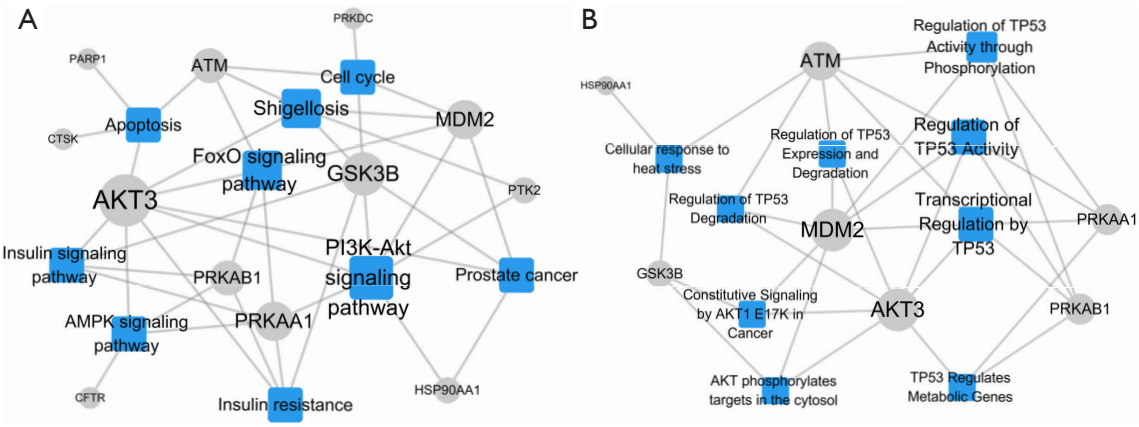

Figure 5 Pathway-target network. (A) Top 9 enriched KEGG pathways-target network; (B) top 9 enriched Reactome pathways-target network. If a target participates in a pathway, there is a link between them. Node size is proportional to its degree. KEGG, Kyoto Encyclopedia of Genes and Genomes.

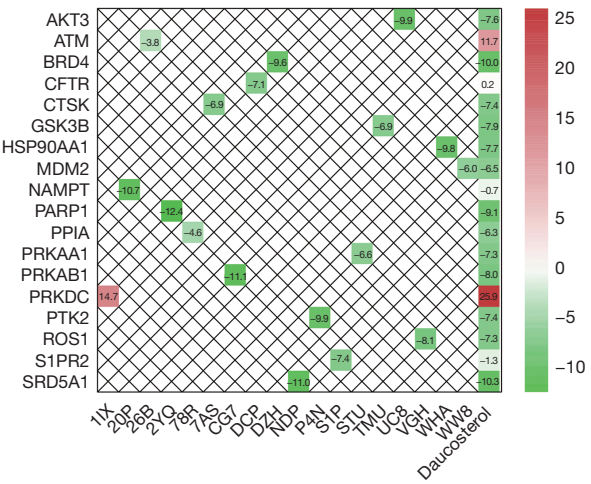

Figure 6 Docking results of between daucosterol and 18 targets. The horizontal axis is the target gene name, and the vertical axis is the target protein co-crystallization small molecules and daucosterol. The numbers in the lattice represent the binding free energy (kcal/mol). The lower the binding energy, the more stable is the ligand-receptor binding conformation (threshold: ≤−5.0 kJ/mol). The PDB_id for targets are as follows: AKT3 (7NH5), ATM (3DDS), BRD4 (6FO5), CFTR (5TFJ), CTSK (5TDI), GSK3B (1Q5K), HSP90AA1 (3WHA), MDM2 (3LBJ), NAMPT (4LVF), PARP1 (7KKN), PPIA (5T9Z), PRKAA1 (6C9H), PRKAB1 (6B1U), PRKDC (7OTY), PTK2 (6YOJ), ROS1 (3ZBF), S1PR2 (7T6B), SRD5A1 (7C83). The PubChem_cid for molecules are as follows: UC8 (156595845), 26B (25138286), DZH (134163707), DCP (65091), 7AS (124037113), TMU (68670561), WHA (25066238), WW8 (24969086), 20P (887836), 2YQ (135565082), 78R (57384849), STU (44259), CG7 (135567398), 1IX (162394489), P4N (11612883), VGH (11626560), S1P (5283560), NDP (5886).

GLU-54, ARG-90, and THR-219 by 3 hydrogen bonds. Daucosterol formed 5 hydrogen bonds with PRO-95, ASP-96, TYR-139, ASN-140, and ASP-144 in BRD4. *PARP1* and daucosterol were bound at TYR-1224 by 2 hydrogen bonds. The *PRKAB1*-daucosterol complex was stabilized by 3 hydrogen bonds at amino acid residue LYS-29, ASN-48, and ARG-83. Daucosterol formed 1 hydrogen bond with SER-203 in *GSK3B*. *HSP90AA1* and daucosterol were bound at ASN-51, GLY-135, and TYR-139 by 3 hydrogen bonds. The sugar ring structure in daucosterol plays a key role in the formation of hydrogen bonds.

Discussion

Relevant studies have shown that daucosterol has anti-tumor potential, but no studies related to MM have been reported. Therefore, in this study, the therapeutic potential and the mechanism of action of daucosterol were evaluated using network pharmacology and molecular docking methods.

To systematically evaluate the therapeutic potential of daucosterol for MM, a network-based RWR algorithm was applied. It adapted a global distance measure to define similarity between genes within this interaction network, which is better suited to capture relationships between drug targets and disease proteins than overlapping genes, direct interactions, or shortest paths between them (44). This step ensures that subsequent analysis is meaningful. It is highly necessary for network pharmacological analysis. In addition, this study innovatively took marketed drugs as the control. The results showed that 13 reported drugs

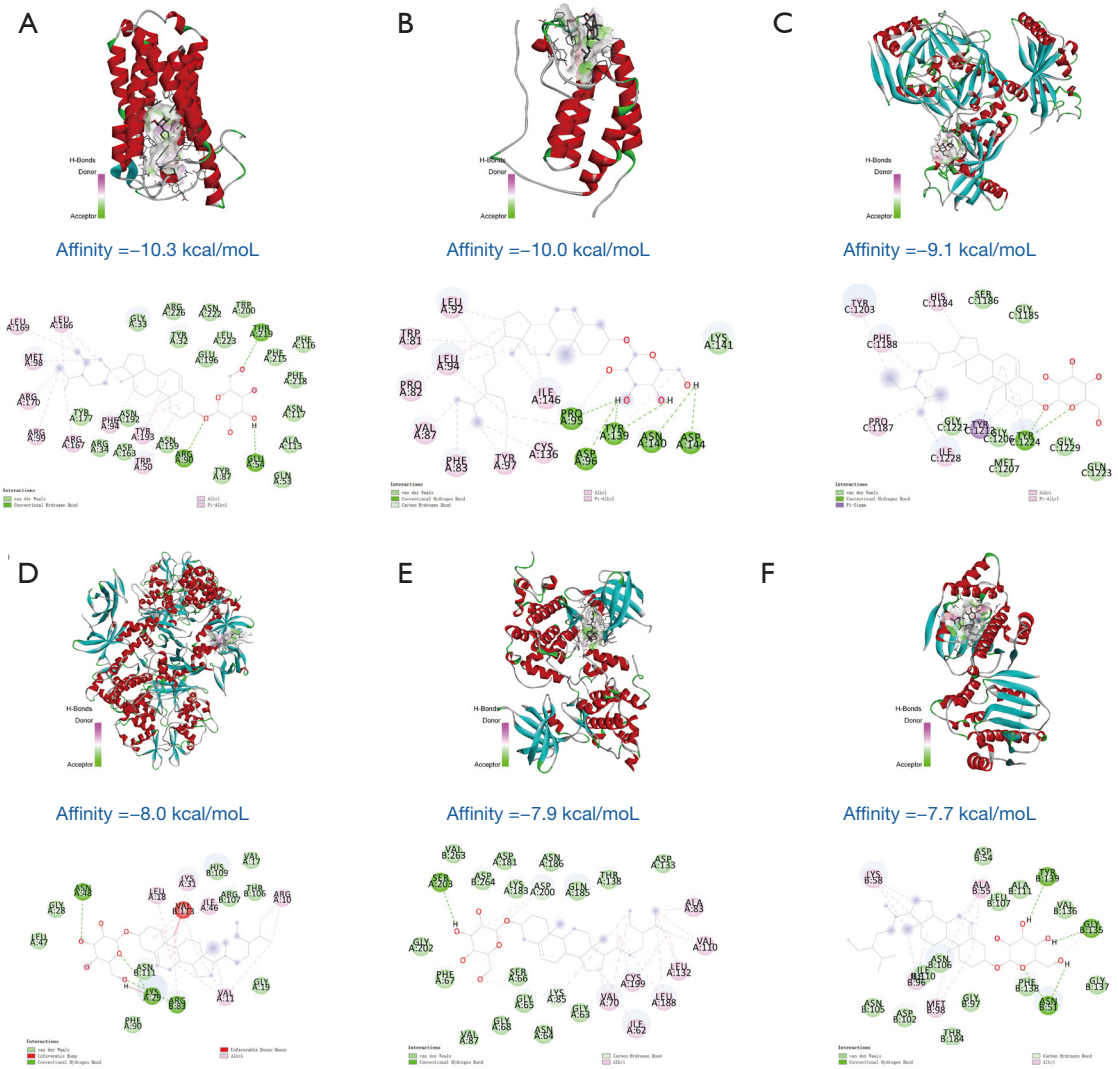

Figure 7 The interaction mode between daucosterol and 6 potential targets. (A) SRD5A1; (B) BRD4; (C) PARP1; (D) PRKAB1; (E) GSK3B; (F) HSP90AA1.

showed significant correlations with MM, which validated the reliability of the method. Among them, bortezomib, a PI with the highest correlation (Z-score =20.5) with MM, is the current first-line treatment drug for MM, which again proves the reliability of this analysis method. The number of candidate targets for drugs varies, which may affect the ranking. Daucosterol and bortezomib, for example, have 35 and 143 candidate targets, respectively. The imbalance of target number may be the main reason for the low ranking of daucosterol, but it does not affect its significant correlation with MM gene.

Pathway enrichment analysis allows us to provide some reference for exploring the mechanism of disease occurrence and the mechanism of drug action from a systematic perspective. KEGG and Reactome pathway analysis showed that pharmacological effect of daucosterol against MM may be mainly through regulating of the FoxO signaling pathway, prostate cancer, PI3K-Akt signaling pathway, insulin resistance, AMPK signaling pathway, and pathways related to the regulation of *TP53*. A multitude of related studies have shown that these pathways are closely related to the occurrence and development of MM (55-60). At present, the mechanism of daucosterol to treat tumors mainly involves the JNK pathway (18), Wnt/β-catenin pathway (14), cell cycle (19), oxidative stress (17), and so on. The high-quality data in this study lay the foundation

and highlight a new direction for the follow-up mechanism research of daucosterol in the treatment of MM.

Through integrated analysis of multiple types of networks, 6 core targets of daucosterol in the treatment of MM were identified, including *HSP90AA1*, *MDM2*, *GSK3B*, *AKT3*, *PRKAA1*, and *PRKAB1*. These targets are at the core position of the PPI network and play an important cross-talking role in multiple signaling pathways. Their intervention can maximize the impact of MM pathophysiology. Research has clearly demonstrated that HSPs might be novel tumor antigens for immunotherapy of myeloma (61). *MDM2* is elevated in MM and is key to MM growth and survival (62). Targeting an MDM2/MYC axis could overcome drug resistance in MM (63). *GSK3β*could activate the β-catenin pathway in MM cells and promotes the malignancy of MM (64,65). *AKT3* overexpression is closely related to MM progression (66). *PRKAB1* participates in the AMPK pathway, and the activation of AMPK pathway is associated with survival of MM cells (67). *PRKAA1* is involved in PI3K-Akt signaling pathway, and the activated PI3K-AKT pathway could accelerate cellular proliferation of MM (68). Studies have shown that daucosterol could significantly increase the levels of *GSK3B*, *PRKAA1*, and *PRKAB1*, and promote the inducting of GSK3-beta phosphorylation and AMPK phosphorylation respectively (69). *GSK3B* plays a key role in the Wnt/β-catenin pathway. Our previous research showed that daucosterol could inhibit cell migration and invasion in hepatocellular carcinoma cells (HCC) cells via the Wnt/β-catenin signaling pathway (14). Therefore, it is necessary to conduct similar studies in MM-related cell or animal models in subsequent studies to explore the mechanism of daucosterol in the treatment of MM.

The reliability of the interaction relationships between 18 predicted anti-MM targets of daucosterol was verified by molecular docking, and the results showed that daucosterol could form stable binding conformations with 13 of them (affinity <–5 kcal/mol). This indicates that the strategy used in this study to obtain the target profiles of compounds is very reliable and can be used by other researchers to obtain the potential target profiles of compounds of interest. The complex formed between daucosterol and *BRD4*, *GSK3B*, *GSK3B*, *CTSK*, *PRKAA1*, *MDM2*, and *PPIA* has a lower binding free energy than the complex formed by the molecules crystallized together with these targets. These results suggest that the binding stability of daucosterol in these targets is better than that of co-crystallized molecules, and it may have better activity than them. The effect of daucosterol on the activity of these targets is worthy of further study. Analysis of the interaction modes between daucosterol and 13 candidate targets suggested that the sugar ring structure in daucosterol was very important and involved in the formation of multiple hydrogen bonds stabilizing conformation with targets. This is an important reminder for the subsequent research on drug development and design modification.

Conclusions

In this study, the therapeutic potential and mechanism of action of daucosterol in the treatment of MM were investigated by network pharmacological and molecular docking technology. On the basis of obtaining high quality therapeutic target profiles of daucosterol and MM gene sets, the therapeutic potential of daucosterol against MM was systematically evaluated through a network-based approach. The pharmacological effect of daucosterol against MM may be mainly through regulation of the FoxO signaling pathway, prostate cancer, PI3K-Akt signaling pathway, insulin resistance, AMPK signaling pathway, and pathways related to the regulation of *TP53*. Key targets involved in multiple pathways include *HSP90AA1*, *MDM2*, *GSK3B*, *AKT3*, *PRKAA1*, and *PRKAB1*. This study reflects the characteristics of pharmacological effects of natural products with "multiple targets and multiple pathways", which lays a foundation for the subsequent in-depth study of daucosterol in the treatment of MM and provides a basis for its application in the clinical treatment of MM.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-456/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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