


# A network pharmacology approach to explore and validate the potential targets of ginsenoside on osteoporosis

International Journal of  
Immunopathology and Pharmacology  
Volume 36: 1–14  
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DOI: 10.1177/03946320221107239  
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## Abstract

**Background:** Osteoporosis (OP) is determined as a chronic systemic bone disorder to increase the susceptibility to fracture. Ginsenosides have been found the anti-osteoporotic activity of in vivo and in vitro. However, its mechanism remains unknown.

**Methods:** The potential mechanism of ginsenosides in anti-osteoporotic activity was identified by using network pharmacology analysis. The active compounds of ginsenosides and their targets associated to OP were retrieved from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, Drug Bank, Pharmmapper, and Cytoscape. The Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis target genes were performed in String, Phenopedia, DisGeNET database, and Metascape software. The protein to protein interaction were created by String database and Cytoscape software. The molecular docking was used to investigate the interactions between active compounds and potential targets by utilizing SwissDock tool, UCSF Chimera, and Pymol software.

**Results:** A total of eight important active ingredients and 17 potential targets related to OP treatment were subjected to analyze. GO analysis showed the anti-osteoporosis targets of ginsenoside mainly play a role in the response to steroid hormone. KEGG enrichment analysis indicated that ginsenoside treats OP by osteoblast differentiation signal pathway. Lastly, the molecular docking outcomes indicated that ginsenoside rh2 had a good binding ability with four target proteins IL1B, TNF, IFNG, and NFKBIA.

**Conclusion:** IL1B, TNF, IFNG, and NFKBIA are the most important targets and osteoblast differentiation is the most valuable signaling pathways in ginsenoside for the treatment of OP, which might be beneficial to elucidate the mechanism concerned to the action of ginsenoside and might supply a better understanding of its anti-OP effects.

## Keywords

Ginsenoside, osteoporosis, network pharmacology, enrichment analysis, validation

Date received: 17 June 2021; accepted: 25 May 2022

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## Introduction

Osteoporosis (OP) is determined as a chronic systemic bone disorder, characterized by low bone mass and microarchitectural degradation of bone tissue, thereby increasing the vulnerability of bone and susceptibility to fracture.<sup>1</sup> A recent report derived from the International Osteoporosis Foundation represents that approximately four million men and 16 million women are affected by OP in the six countries of the European Union.<sup>2</sup> Hip fractures and vertebral fractures are the most common fracture associated with bone fragility, which have led to extensive morbidity, mortality, and other adverse health consequences. It was estimated that approximately 2.7 million hip fractures happened due to OP in 2010 worldwide.<sup>1</sup> Pharmacological therapies, including Bisphosphonates, Denosumab, Raloxifene, Teriparatide, and Abaloparatide, have been applied to reduce the fracture risk in individual osteoporotic patient.<sup>1,3</sup> However, these medications also brought about some adverse events, such as upper gastrointestinal adverse reactions, skin rash, breast pain and muscle cramps, and so on.<sup>1</sup> Recently, scientists have found that traditional Chinese medicines (TCM) have great potential in preventing and treating OP on account of good curative effect and minimal side effects. *Cistanche tubulosa*,<sup>4</sup> Muscone,<sup>5</sup> Xian-Ling-Gu-Bao prescription,<sup>6</sup> and *Morinda officinalis* have been explored to be beneficial for osteoporosis with different mechanisms. Although these TCMS have been examined to be potential treatment efficacy for OP, the best one is not yet found.

As the primary pharmacologically active ingredients of the ginseng root, ginsenosides are a type of sterol compound including a structure with four-ring and a carbohydrate steroidal body.<sup>7</sup> To date, the researchers have isolated and identified a total of 40 structurally diverging ginsenosides from the *P. ginseng* root (Table 1). Several studies have explored the anti-osteoporotic activity of ginsenosides in vivo, in vitro, and rat.<sup>8–10</sup> However, although some progress has been achieved on the research of OP with ginsenosides, we are still lack of systematic, in-depth, and comprehensive understanding on the intrinsic interaction between potential targets and signaling pathways involved this. Network pharmacology is a scientific research method that has been extensively applied in drug study based on its strong, efficient, and holistic function. Here, the potential molecular targets and signaling pathways on ginsenosides involving the OP treatment will be fully characterized, and the potential related mechanism will also be clearly described by establishing a network of ginsenosides and OP target interrelation. The workflow on this study is shown the Figure 1.

## Materials and methods

### Screening of ginsenosides pharmacologically active ingredient

Ginsenosides are kinds of active substances of traditional Chinese medicine ginseng. We obtained the drug active component information by searching the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <http://lsp.nwu.edu.cn/index.php>)<sup>11</sup> based on the keyword: renshen (ginseng). Absorption, Distribution, Metabolism, Excretion<sup>12</sup> properties of each active component of medicinal herbs could be derived from the TCMSP database, which contains related information, such as Oral Bioavailability (OB), Drug-Likeness (DL), Half-Life (HL), and so on.

First, we gained ginsenosides from all active substances results of ginseng by TCMSP. Then, the ingredients of ginsenosides with  $OB \geq 30\%$  and  $DL \geq 0.7$  were selected as main research subjects in this study. We also acquired the three-dimension (2D) chemical structures of these selected ginsenosides from the TCMSP database in mol2 format.

### Prediction of potential target proteins for ginsenosides

The TCMSP database has given some potential target proteins of each active component. Some targets results are supported by Drug Bank database (<https://www.drugbank.ca/>).<sup>13</sup> We first stored these results as part of potential targets of selected ginsenosides. Second, Pharmmapper database (<http://www.lilab-ecust.cn/pharmmapper/>) were used to identify other potential human target proteins for the ginsenosides. Pharmmapper could identify potential target candidates for a given small molecule structure by a pharmacophore mapping approach. Three dimensions (3D) chemical structure of each selected ginsenoside was uploaded to the Pharmmapper with default parameters of database. 300 potential target candidate results of each ginsenoside were downloaded after handling.<sup>14</sup>

Third, we chose human proteins from Pharmmapper results as target candidates. Together with results of TCMSP in the first step, we got the potential target proteins for selected ginsenosides. Then we used Cytoscape software to visualize the relationship between selected ginsenosides and potential targets.<sup>15</sup>

### Screening the potential target genes related to osteoporosis

Phenopedia database (<http://www.hugenavigator.net/HuGENavigator/startPagePhenoPedia.do>) is an application based

**Table 1.** The 40 structurally divergent ginsenosides are isolated and identified from the *P. ginseng* root.

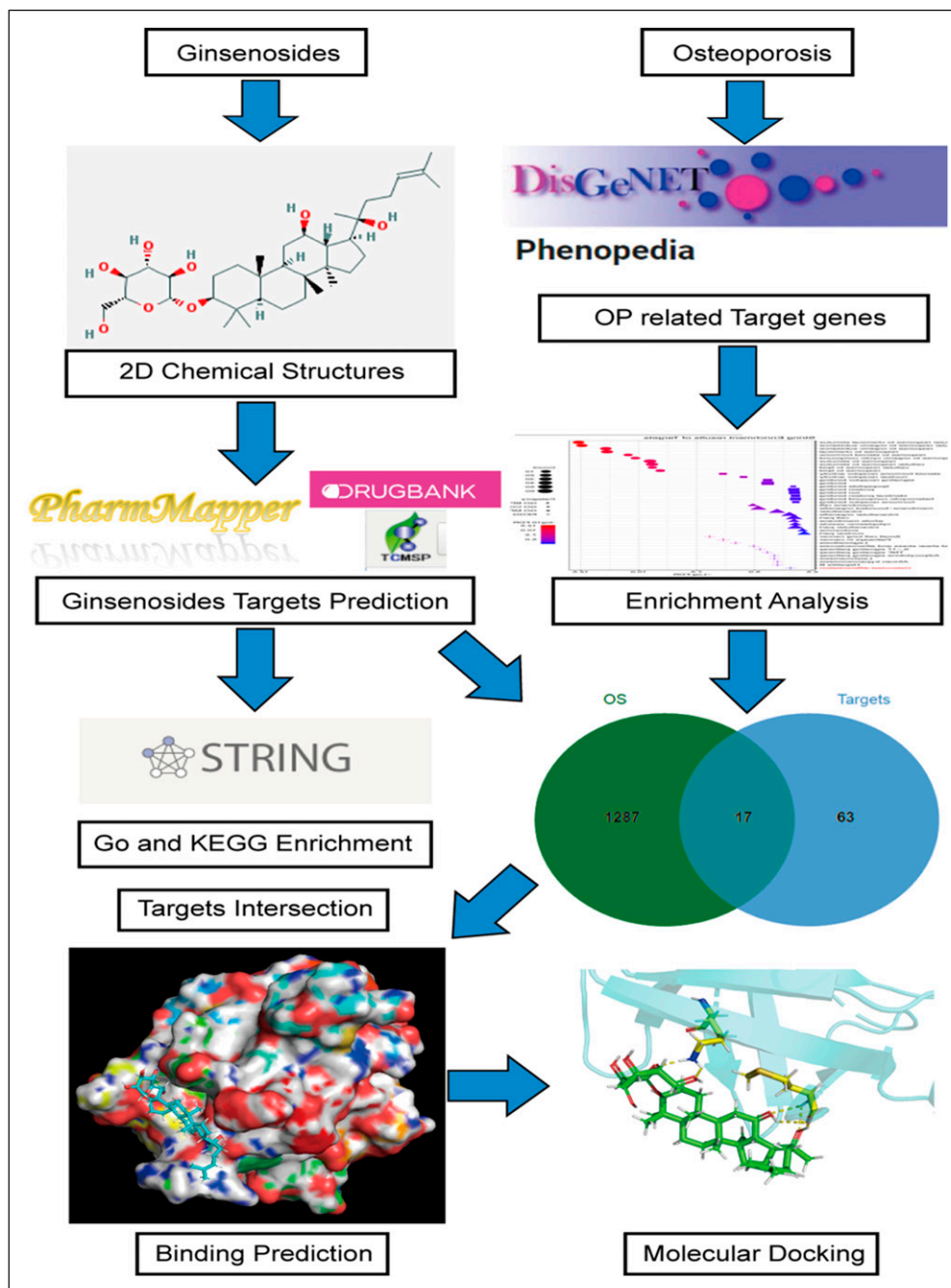
Mol ID	Molecule name	MW	AlogP	Hdon	Hacc	OB (%)	Caco-2	BBB	DL	FASA-
MOL005155	Ginsenoside Ro <sub>qt</sub>	455.77	5.74	1	3	17.62	0.38	0.05	0.76	0.04
MOL005279	Ginsenoside-Rh1	638.98	2.87	7	9	3.86	-1.17	-2.14	0.57	0.25
MOL005280	Ginsenoside-Rh1 <sub>qt</sub>	476.82	4.62	4	4	20.13	-0.02	-0.9	0.78	0.24
MOL005281	20(S)-Ginsenoside-Rh1	638.98	2.87	7	9	3.94	-1.1	-2.04	0.57	0.25
MOL005282	20(S)-Ginsenoside-Rh1 <sub>qt</sub>	476.82	4.62	4	4	20.13	0.17	-0.56	0.78	0.24
MOL005283	20(S)-ginsenoside-Rg2	785.14	2.02	9	13	10.09	-1.94	-2.78	0.26	0.23
MOL005286	20(R)-ginsenoside Rg2	785.14	202	9	13	10.09	-1.96	-2.95	0.26	0.27
MOL005287	20-(S)-Ginsenoside-Rg3	785.14	2.3	9	13	13.69	-1.65	-2.47	0.22	0.24
MOL005288	20-(S)-Ginsenoside-Rg3 <sub>qt</sub>	460.82	5.79	3	3	29.69	0.55	-0.12	0.77	0.24
MOL005300	6'-Malonylginsenoside Rd1	1195.51	-1.03	15	26	6.7	-4.21	-5.39	0.03	0.29
MOL005301	6'-Malonylginsenoside Rd1 <sub>qt</sub>	460.82	5.79	3	3	29.69	0.56	-0.13	0.77	0.25
MOL005323	Ginsenoside La	783.12	1.84	8	13	17.74	-1.9	-3.25	0.14	0.24
MOL005324	Ginsenoside La <sub>qt</sub>	458.8	5.33	2	3	15.7	0.65	0.04	0.78	0.24
MOL005325	Ginsenoside Ro	957.24	1.23	11	19	1.98	-2.86	-3.92	0.05	0.26
MOL005326	Ginsenoside-Ra0	1271.62	-2.94	18	28	7.3	-5.56	-6.76	0.01	0.22
MOL005328	Ginsenoside-Ra1	1211.56	-1.92	16	26	7.09	-4.51	-6.41	0.02	0.22
MOL005329	Ginsenoside-Ra2	1211.56	-1.92	16	26	7.62	-4.43	-6.28	0.02	0.24
MOL005330	Ginsenoside-Ra3	1241.59	-2.43	17	27	7.19	-4.67	-6.3	0.02	0.26
MOL005331	Ginsenoside Rb1	1109.46	-1.2	15	23	6.24	-3.99	-5.6	0.04	0.23
MOL005333	Ginsenoside-Rb2	1079.43	-0.69	14	22	6.02	-3.92	-5.43	0.04	0.26
MOL005336	Ginsenoside-Rc	1079.43	-0.69	14	22	8.16	-3.97	-5.69	0.04	0.25
MOL005338	Ginsenoside Re	947.3	0.27	12	18	4.27	-3.2	-4.39	0.12	0.25
MOL011400	Ginsenoside rf	801.14	1.13	10	14	17.74	-2.23	-3.35	0.24	0.27
MOL005342	Ginsenoside-Rg3	785.14	2.3	9	13	17.75	-2.02	-2.75	0.22	0.25
MOL005343	Ginsenoside-Rg3 <sub>qt</sub>	460.82	5.79	3	3	29.69	0.31	-0.58	0.77	0.26
MOL005344	Ginsenoside rh2	622.98	4.04	6	8	36.32	-0.51	-1.38	0.56	0.24
MOL005346	Ginsenoside-Rh3 <sub>qt</sub>	442.8	6.76	2	2	13.09	0.97	0.42	0.76	0.26
MOL005347	Ginsenoside-Rh4	620.96	3.84	6	8	5.22	-0.73	-1.65	0.6	0.25
MOL005348	Ginsenoside-Rh4 <sub>qt</sub>	458.8	5.59	3	3	31.11	0.5	-0.18	0.78	0.25
MOL005349	Ginsenoside-Rs1	1121.47	-0.31	13	23	6.27	-3.69	-5.37	0.04	0.24
MOL005350	Ginsenoside-Rs2	1121.47	-0.31	13	23	8.14	-4.03	-5.58	0.04	0.24
MOL005361	Malonylginsenoside Rc	1165.48	-0.52	14	25	7.84	-4.27	-5.65	0.03	0.25
MOL005362	Malonylginsenoside Rc <sub>qt</sub>	460.82	5.79	3	3	29.69	0.45	-0.44	0.77	0.25
MOL005363	Malonylginsenoside Rd	1033.35	0.71	12	21	8.84	-3.72	-4.76	0.07	0.28
MOL005364	Malonylginsenoside Rd <sub>qt</sub>	460.82	5.79	3	3	29.69	0.47	-0.23	0.77	0.26
MOL005372	Notoginsenoside R2	771.11	1.64	9	13	17.74	-2.22	-3.12	0.28	0.25
MOL005373	Notoginsenoside R2 <sub>qt</sub>	476.82	4.62	4	4	20.13	0.2	-0.49	0.78	0.24
MOL005374	Notoginsenoside R6	963.3	-0.62	13	19	4.7	-3.46	-4.39	0.12	0.24
MOL005400	Ginsenoside Rg5	767.12	3.31	8	12	6.15	-1.92	-2.83	0.23	0.26
MOL005401	Ginsenoside Rg5 <sub>qt</sub>	442.8	6.8	2	2	39.56	0.88	0.21	0.79	0.24

on web page with persistently renewed from PubMed, which is able to explore the literatures associated with human genes and diseases.<sup>16</sup> In this analysis, the keyword “Osteoporosis” was firstly searched in the Phenopedia database to obtain the target genes related to OP. DisGeNET database (<http://www.disgenet.org/>) is a standardized intellectual management platform based on multiple scientific literatures, which covers a full range of human diseases and related genes.<sup>17</sup> The target genes associated with OP were also explored in this database. Then, we took the union results gained from both Phenopedia and DisGeNET databases as the potential

target genes of OP. The jvenn tool<sup>18</sup> was used to cross-validate the ginsenoside target proteins and OP-related potential target genes to procure possible target genes of ginsenoside treating OP.

#### Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis for target proteins related to osteoporosis

For the OP target proteins of selected ginsenosides we obtained above, we put to use the Search Tool for the



**Figure 1.** Flowchart of the systematic procedure to investigate the mechanisms of ginsenosides in the treatment of osteoporosis.

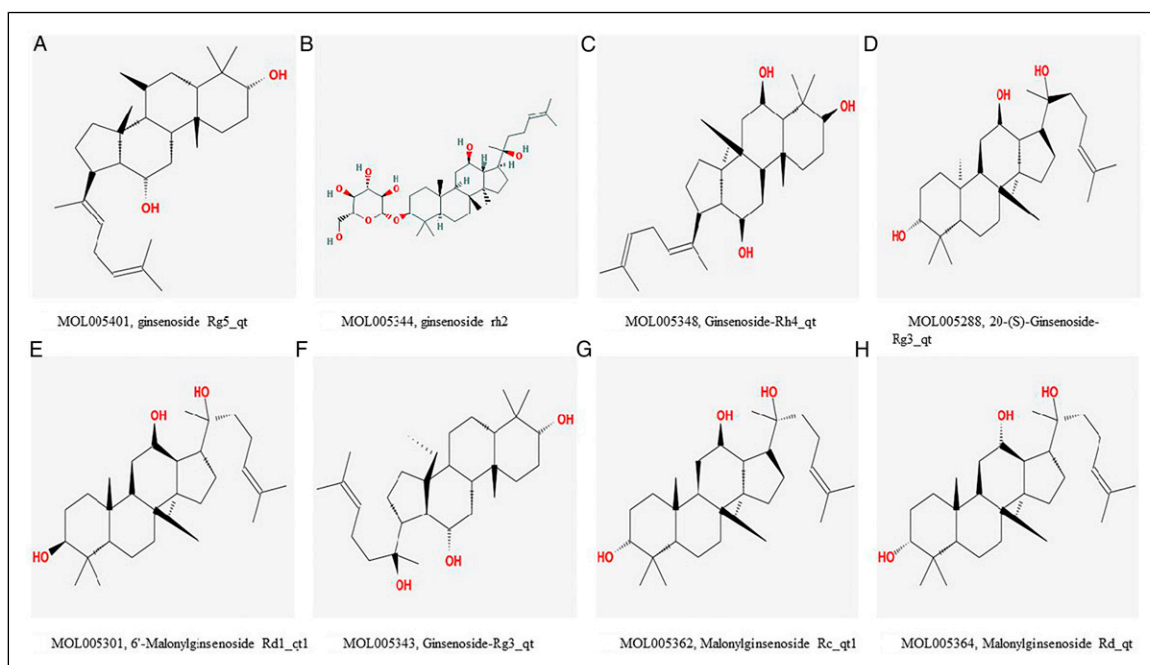
Retrieval of Interacting Genes (String) database (<https://string-db.org/>) to perform Gene ontology (GO) (Biological Process (BP), Cellular Component (CC), and Molecular Function (MF)) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis by default analysis

parameters.<sup>19</sup> For the analysis results harvested from String, we made use of R language (version 4.0.0) to visualize them in the form of bubble charts. In order to ensure the visualization effect, we only visualized the top 10 items of GO and KEGG enrichment pathway. For pathways related to

**Table 2.** The eight active ingredients of ginsenosides obtained for traditional Chinese medicine systems pharmacology database and analysis platform (oral bioavailability  $\geq 30\%$ ; drug-likeness  $\geq 0.7$ ).

Mol ID	Molecule name	MW	AlogP	Hdon	Hacc	OB (%)	Caco-2	BBB	DL	FASA-	HL
MOL005401	Ginsenoside Rg5_qt	442.8	6.8	2	2	39.56	0.88	0.21	0.79	0.24	5.65
MOL005344	Ginsenoside rh2	622.98	4.04	6	8	36.32	-0.51	-1.38	0.56	0.24	11.08
MOL005348	Ginsenoside-Rh4_qt	458.8	5.59	3	3	31.11	0.5	-0.18	0.78	0.25	6.97
MOL005288	20-(S)-Ginsenoside-Rg3_qt	460.82	5.79	3	3	29.69	0.55	-0.12	0.77	0.24	
MOL005301	6'-Malonylginsenoside Rd1_qt1	460.82	5.79	3	3	29.69	0.56	-0.13	0.77	0.25	
MOL005343	Ginsenoside-Rg3_qt	460.82	5.79	3	3	29.69	0.31	-0.58	0.77	0.26	
MOL005362	Malonylginsenoside Rc_qt1	460.82	5.79	3	3	29.69	0.45	-0.44	0.77	0.25	
MOL005364	Malonylginsenoside Rd_qt	460.82	5.79	3	3	29.69	0.47	-0.23	0.77	0.26	

Mol ID: molecular identity; MW: molecular weight; AlogP: lipid-water partition coefficient; Hdon: Number of Hbond donor; Hacc: Number of Hbond recipient; OB: Oral bioavailability; Caco-2: Intestinal epithelial permeability; BBB: Blood brain barrier; DL: Drug likelihood; HL: Drug half-life.

**Figure 2.** The 2D chemical structures of the eight ginsenoside active components.

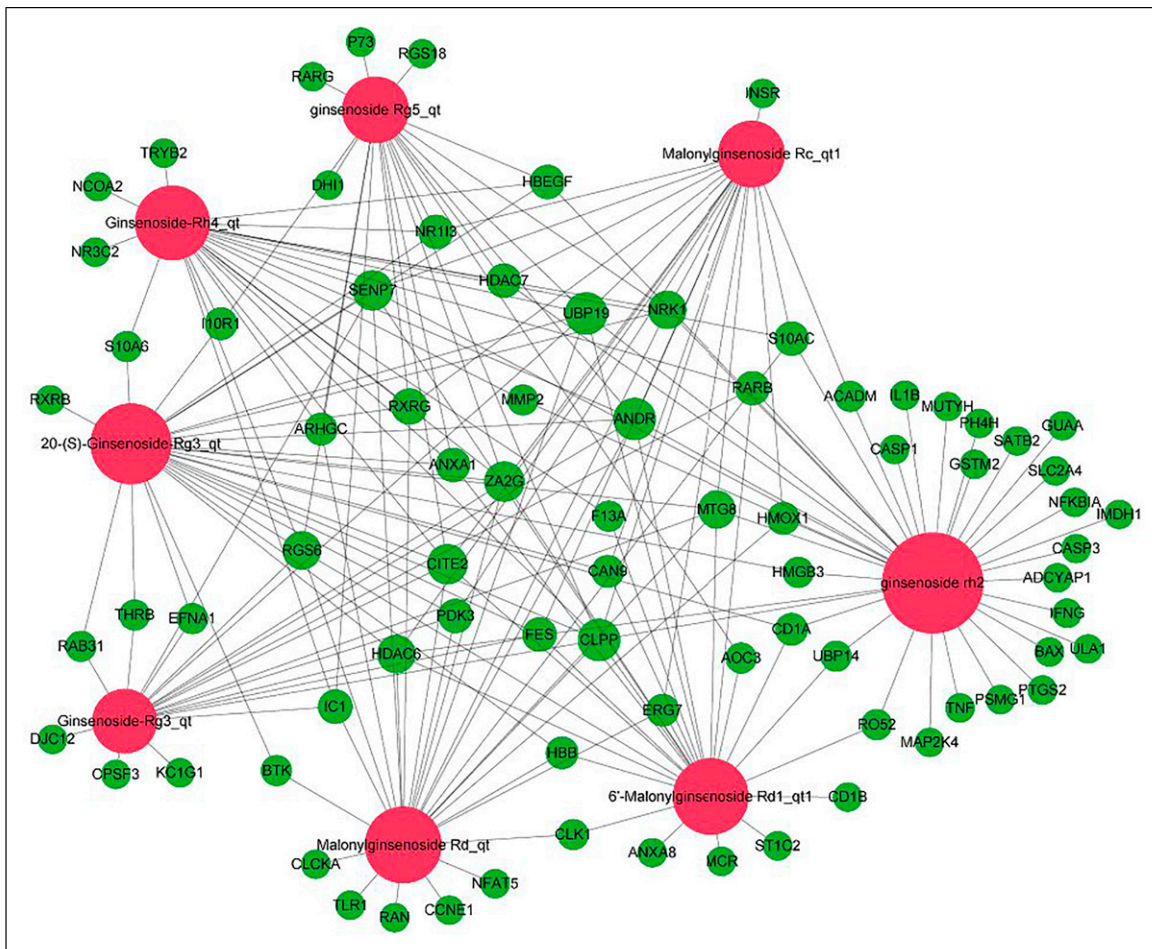
osteogenesis, we further applied the R package “pathview” to visualize their pathways and related targets.<sup>20</sup>

### Protein-protein interaction analysis for key target proteins related to osteoporosis treatment

To explore the interaction between the protein targets of ..... and identify the key proteins, the overlapping genes were imported into the STRING 11.0 database (<https://string-db.org/>), and the species was set to “*Homo sapiens*” after “Multiple Proteins” was selected; the other parameters were defaulted (combined score  $>0.4$ , medium confidence). The obtained protein interaction results were saved in TSV

format. The files’ node 1, node 2, and combined score information were retained and imported into Cytoscape software to draw the interaction network. The Network Analyzer tool in Cytoscape software was used to analyze the network. The node size and color were set to reflect the size of the degree value to obtain the final protein-protein interaction (PPI) network. Then we made use of the MCODE plug-in of Cytoscape to analyze the key sub-network of the obtained PPI network to study the important topological sub-network structure in the entire network.<sup>21</sup> The analysis parameters of MCODE were set to degree cut off = 2, node score cut off = 0.2, Max depth = 100, k score = 2, and MCODE score  $>5$ .





**Figure 3.** Network of target proteins in the eight ginsenoside active components.

### *Molecular docking verification of ginsenoside to target proteins related to osteoporosis treatment*

In order to elucidate how the ginsenosides bind to target proteins to exert the therapeutic efficacy, we used the SwissDock tool to make docking predictions. Four proteins (IL1B, TNF, IFNG, and NFKBIA) with the most connections in the [Figure 7](#), which are the major factors in the osteoclast differentiation pathway ([Figure 5](#)), were selected to make the binding prediction. We obtained the Protein Data Bank (PDB) identifications (IDs)<sup>22</sup> of key target proteins related to OP treatment from the PDB database (PDB, <http://www.pdb.org/>). With the 3D-structure in mole format of selected ginsenoside, target proteins were uploaded to SwissDock website to make docking predictions.<sup>23</sup> The model with minimal G scores was considered as the most excellent docking model. For the optimal prediction results we got, we took advantage of UCSF Chimera and Pymol software to visualize them.<sup>24</sup> In order to verify the prediction results of Swissdock, we downloaded the three-digit structure files of the four proteins from the PDB

database and used AutoDock software (V4.2.6) to do molecular docking of ginsenosides and proteins.

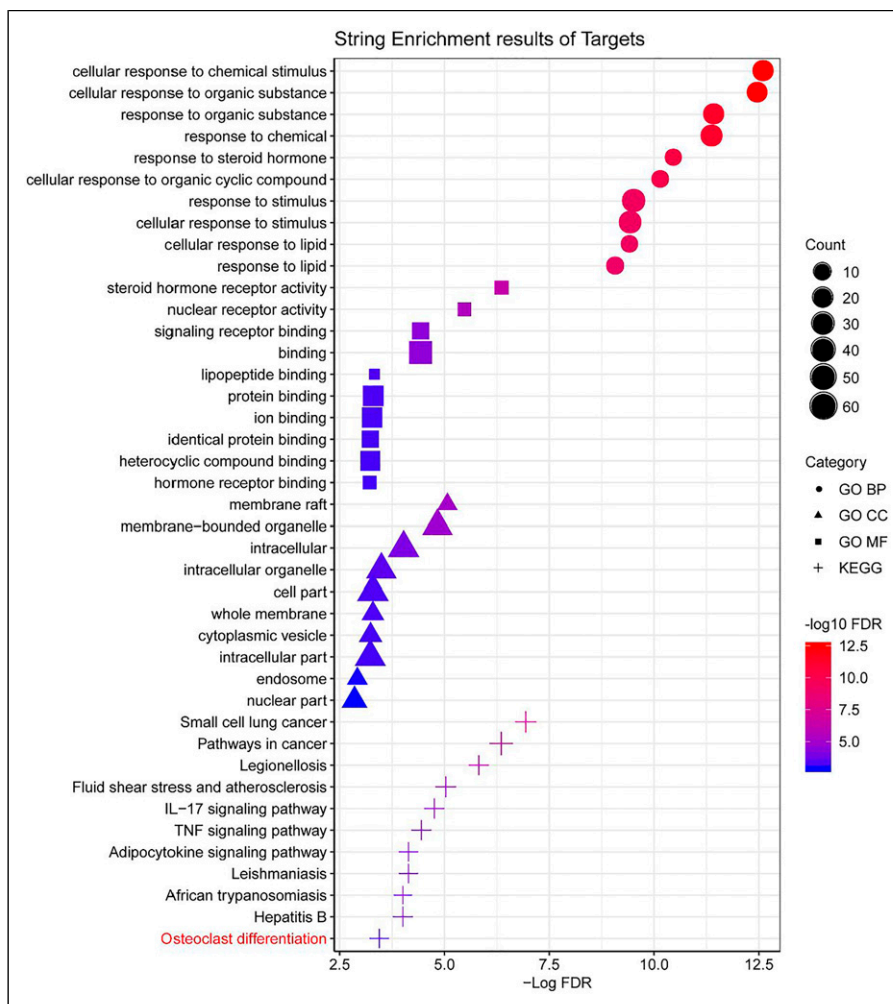
## **Results**

### *Pharmacologically active ingredients of ginsenosides and 2D chemical structure*

We obtained 190 TCM active ingredients when we searched in TCMSP database on account of keyword “renshen (ginseng).” According to filter threshold, a total of eight important active ingredients of ginsenosides were obtained, which were listed in [Table 2](#). Then, the 2D chemical structures of these eight ginsenoside active components were acquired from TCMSP. They are showed in [Figure 2](#).

### *Prediction of target proteins in the eight ginsenoside active components*

In the TCMSP database, only ginsenoside rh2 and Ginsenoside-Rh4\_qt got the predicted target proteins. Therefore, we explored



**Figure 4.** String gene ontology and KEGG enrichment results of the target proteins. (●) Biological Processes, (■) Molecular Function, (▲) Cellular Components, and (+) KEGG. Note: Only the top 10 items are listed in the figures above. “FDR” is on behalf of the number and ratio of the candidate target genes located in a pathway. A higher FDR suggests a greater degree of enrichment. A larger size of a dot indicates a greater deal of target genes belonging to the pathway. Various colors of a dot implicates diverse  $p$  values. KEGG: Kyoto Encyclopedia of Genes and Genomes.

the target proteins of these eight ginsenoside active components in the Phammapper database. A total of 66 and 14 target proteins were secured in the Phammapper and TCMSP database, respectively. Finally, a network with 80 target proteins and eight ginsenosides was constructed (Figure 3). The larger the red circle, the more connections. As we can see, ginsenoside rh2 contains the most connections.

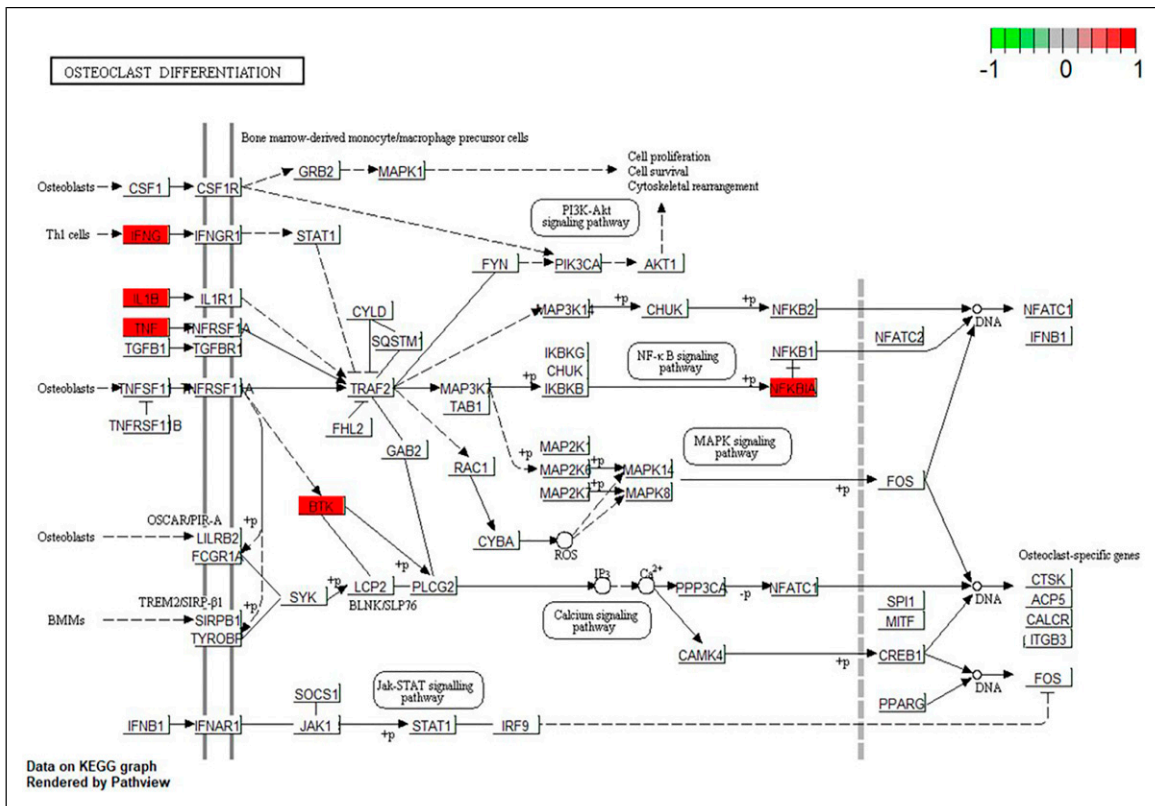
### The potential target of ginsenoside treating osteoporosis

The cross-validation was performed between OP-related genes and ginsenoside target protein. Then, we took the cross-validated intersection as the possible targets for ginsenoside in the treatment of OP. As seen in the Figure 4, a

total of 1304 OP-related genes were obtained, and a total of 17 overlapping genes were achieved after cross-validation with 80 ginsenoside target proteins. These 17 overlapping genes are *IL1B*, *BAX*, *MMP2*, *HBB*, *TNF*, *IFNG*, *RXRG*, *RXRB*, *RARB*, *THRB*, *CASPI*, *CASP3*, *SLC2A4*, *RARG*, *HMOX1*, *NFKBIA*, and *NCOA2*. Except *BTK*, four of the five genes involved in the osteoclast differentiation pathway are contained in these 17 overlapping target genes.

### Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment results of target proteins of ginsenosides

To further explore the various mechanisms of the anti-osteoporotic activity of ginsenosides, we performed a GO



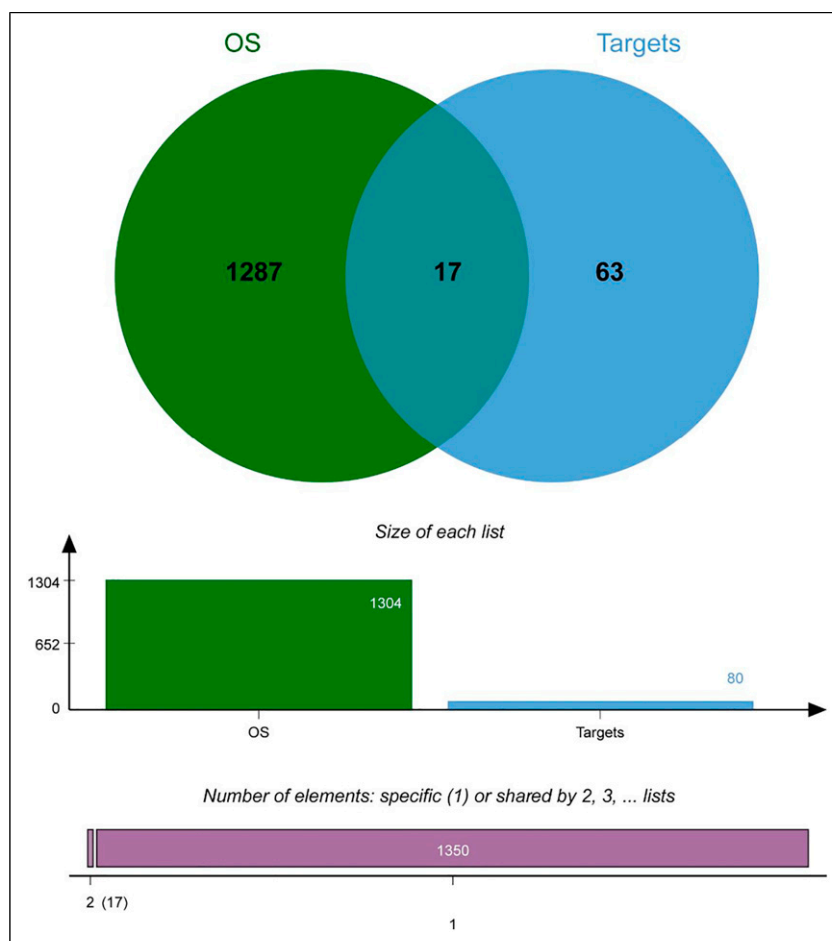
**Figure 5.** The ginsenoside target proteins (red) in the osteoclast differentiation pathway.

and KEGG enrichment analysis (BP, MF, and CC) of the 17 target genes. The GO and KEGG pathway enrichment analysis of target proteins in these eight ginsenosides were performed in the String database. The results with the top 10 enriched BP terms, MF terms, and CC terms are shown as bubble chart in the Figure 5. The top five BP terms were as follows: (1) cellular response to chemical stimulus, (2) cellular response to organic substance, (3) response to organic substance, (4) response to chemical stimulus and (5) response to steroid hormone. In the MF terms, the top five terms are (1) steroid hormone receptor activity, (2) nuclear receptor activity, (3) signaling receptor activity, (4) binding, and (5) lipopeptide binding. The top five CC terms were as follows: (1) membrane raft, (2) membrane-bounded organelle, (3) intracellular, (4) intracellular organelle, and (5) cell part. Then, in the KEGG enrichment analysis, the tumor and inflammation related biological pathways are the primary pathways concerned to our target proteins, except osteoclast differentiation pathway (Figure 5). Finally, we found five target proteins participated in the osteoclast differentiation pathway, including BTK, IFNG, IL1B, NFKBIA, and TNF. The completed pathway chart was showed in the Figure 6.

### Protein-protein interaction network of anti-osteoporosis targets of ginsenosides

A mature complicated algorithm called MCODE is used by Metascape to automatically extract target complexes embedded in the large network.<sup>25</sup> It provides dense inter-actome neighborhoods and the interpretation of these biological enrichment pathways. The 17 key anti-OP target proteins were used to perform PPI analysis in the String database. Then, the PPI network obtained above was further analyzed in the plug-in MCODE of Cytoscape to construct a second network. There were 16 nodes in this PPI network, beside HBB (Figure 7(a)). Nevertheless, in the PPI sub-network, the important nodes (genes) became 14 ones (Figure 7(b)), including *IL1B*, *MMP2*, *TNF*, *IFNG*, *RXRG*, *RXRB*, *RARB*, *THRB*, *CASP1*, *CASP3*, *RARG*, *HMOX1*, *NFKBIA*, and *NCOA2*, beside *BAX*, *HBB*, and *SLC2A4*. Therefore, the potential key targets were reduced to 14 ones. In the PPI network, TNF and IL1B were shown to have the most interactions with other ginsenoside targets in the cluster, indicating that ESR1 and AKT1 played an important role in connecting other nodes.





**Figure 6.** The venny diagram of ginsenosides and osteoporosis intersection target genes. Note: 1287 OP non-intersection potential targets (left), 17 OP-ginsenoside intersection potential targets (middle) and 63 ginsenoside non-intersection potential targets (right).

### Prediction of ginsenosides binding to target proteins associated with osteoporosis

The SwissDock database was utilized to predict the binding of ginsenosides and target proteins related to OP. We chose one as the representative drug from the eight main ginsenoside active ingredient with the largest MW, the longest drug HL, and the most target proteins, which was ginsenoside rh2 (MOL005344) and was currently the main ginsenoside drug on the market. Prediction analysis of four target proteins IL1B, TNF, IFNG, and NFKBIA binding to ginsenoside rh2 was performed. The results with optimal docking are shown in Figures 8 and 9.

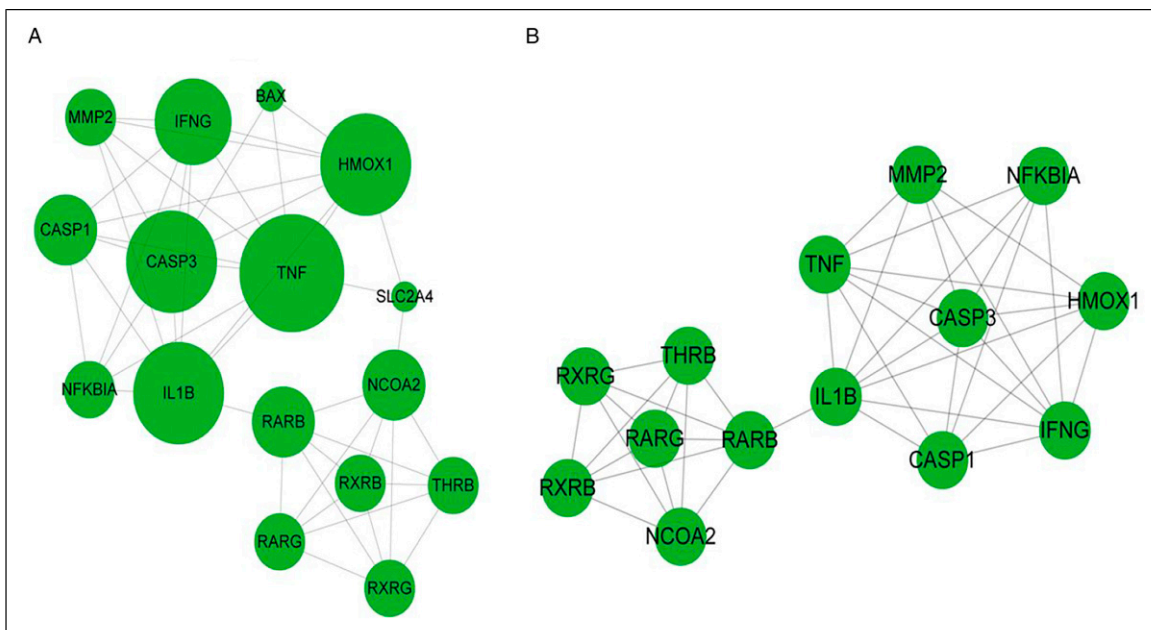
### Validation of binding prediction

Through the validation in the AutoDock software (V4.2.6), two of the four proteins got the last valuable outcomes, which are IFNG and IL1B, seeing Figures 10 and 11. In the Figure 10, the ginsenoside binds to the Pheylalanine 201

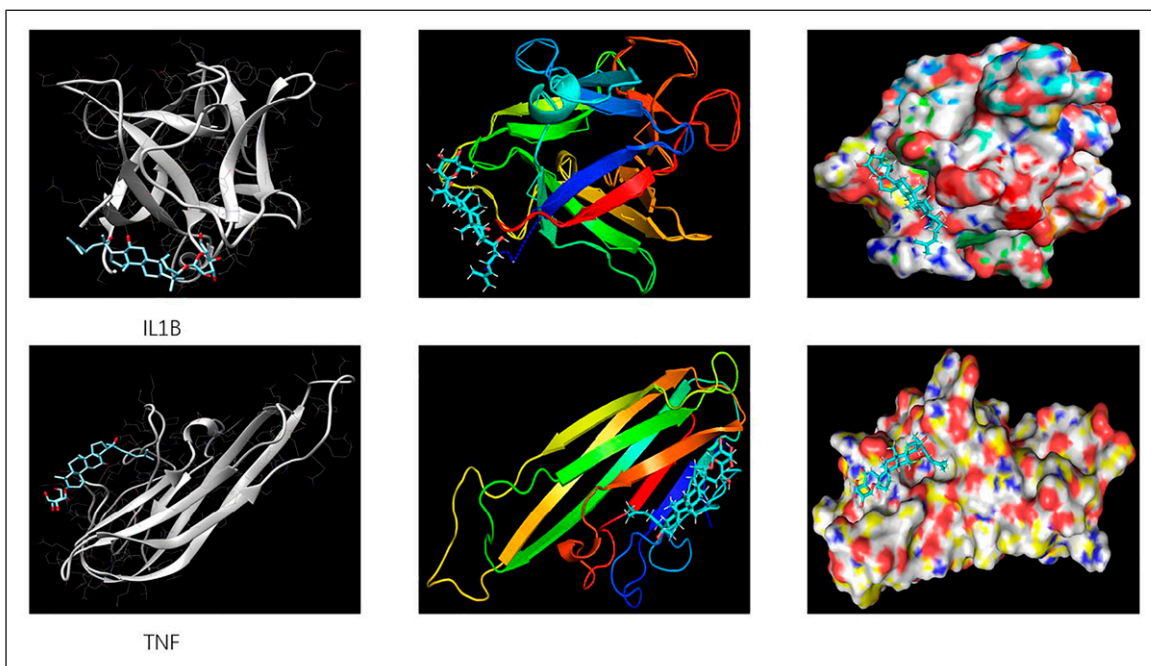
residue (F201), Asparagines 205 residue (N205), Lysine 208 residue (K208), Aspartic acid 41 residue (D41), and Serine 40 residue (S40) of NFKBIA. As shown in Figure 11, the ginsenoside binds to the Lysine 103 residue (K103) and the methionine 148 residue (M148) of the IL1B. These showed that the ginsenoside could interact with these two proteins to play the therapeutic effect in anti-osteoporotic.

### Discussion

Plenty of osteoporosis-associated morbidities have been on the rise in women older than 55 years and men older than 65 years. OP has become a common disorder that has greater potential to threaten the elderly health. Many previous literatures have reported that oxidative stress response, deficiency of estrogen, bone immune disorder, chronic inflammatory reaction, bone marrow microcirculation dysfunction, and glucocorticoid-induced bone loss may be contributed to the pathogenesis of OP.<sup>26,27</sup> Network pharmacology has been broadly used to study drug and



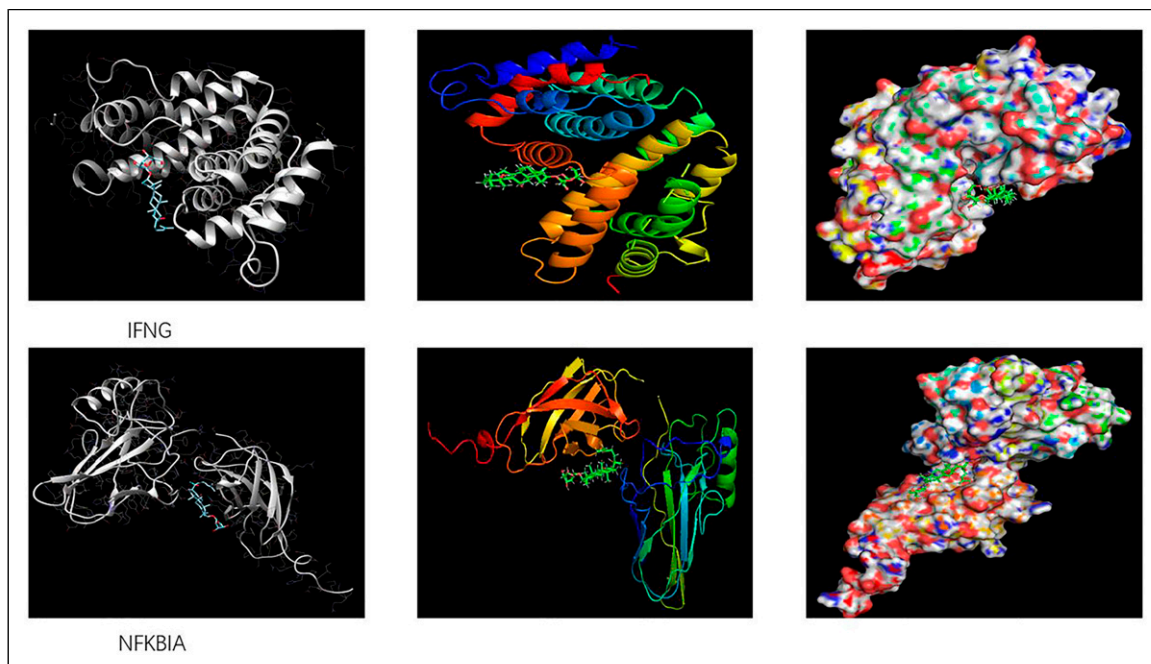
**Figure 7.** PPI network of the anti-osteoporosis target proteins (a) The PPI network constructed using String and Cytoscape . (b) The cluster generated from (a). Node size is proportional to the degree of interaction. PPI: Protein-protein interaction.



**Figure 8.** Prediction of ginsenoside rh2 binding to *IL1B* and *TNF* associated with osteoporosis.  $\Delta G = -8.17$  kcal/mol, up plots;  $\Delta G = -7.23$  kcal/mol, down plots. Note: The large structures like a string is the target protein, the small is ginsenoside rh2. The left plot is made by Chimera software; the middle and right plots are drawn by Pymol software.

relevant disease and made full sense of drug research due to big data analysis.<sup>28</sup> In the clinical practice, ginsenosides have been widely utilized to treat different kinds of disease because of good effectiveness and low side effects. In this

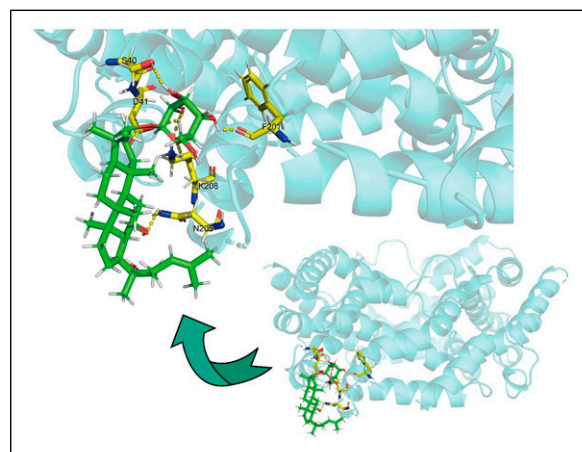
study, the network pharmacology was conducted to explore the potential key targets and mechanisms associated with OP treatment by building and analyzing drug target networks, enrichment analysis.



**Figure 9.** Prediction of ginsenoside rh2 binding to *IFNG* and *NFKBIA* associated with OP.  $\Delta G = -7.55$  kcal/mol, up plots;  $\Delta G = -8.36$  kcal/mol, down plots. Note: The large structures are ginsenoside rhs, the small is target protein. The left plot is made by Chimera software; the middle and right plots are drawn by Pymol software.

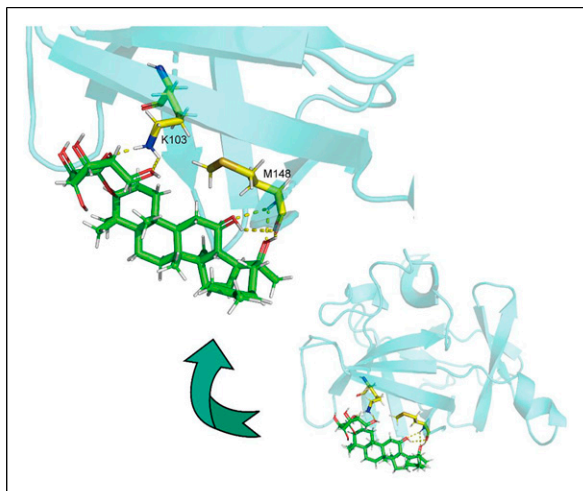
### The potential key targets

Fourteen potential key targets were obtained by cross-validation of intersection and PPI network analysis, which are *IL1B*, *MMP2*, *TNF*, *IFNG*, *RXRG*, *RXR*, *RARB*, *THRB*, *CASP1*, *CASP3*, *RARG*, *HMOX1*, *NFKBIA*, and *NCOA2*. All of these target genes or proteins were considered to be associated with ginsenosides treating OP, some of which have been reported in the recent literatures. However, *IL1B*, *TNF*, *IFNG*, and *NFKBIA* were most important target proteins because of involving in osteoclast differentiation. *IL1B* was detected as an increased inflammation-relevant gene of OP in Kashin-beck disease patients, and was regulated by ginsenoside rhs in treating idiopathic pulmonary fibrosis.<sup>29,30</sup> *MMP2* has been showed that might be a new possible therapeutical target in precaution and treatment of glucocorticoid-related OP.<sup>31</sup> *TNF- $\alpha$*  and *CASP3* expression were regulated to alleviate OP by *Sinomenii Caulis* extract.<sup>32</sup> A recent Cis-eQTL analysis have elucidated that *IFNG* expression was positively related to OP and abdominal obesity.<sup>33</sup> *RARB* was examined to be a bone mineral density loci by genome-wide association studies in Koreans.<sup>34</sup> High glucose induced *caspase-1* and *IL-1B* activation and inhibition of efferocytosis in osteoclast-mediated diabetic osteoporosis.<sup>35</sup> *Fufang Lurong Jiangu Capsule* significantly alleviating oxidative stress in osteoblasts induced by *Nrf2/HMOX-1* axis might provide new perception to the



**Figure 10.** Molecular docked structure of *IFNG*-ginsenoside complex. Docking was performed using AutoDock software (V4.2.6) and various amino acid interactions are mentioned in the respective passage. The larger helical lamellar structure is *IFNG* protein and the smaller hexagon is ginsenoside.

treatment of osteoporosis.<sup>36</sup> And, ginsenoside rb3 defends cardiomyocytes against oxidative injury via activation of *PERK/Nrf2/HMOX1* axis.<sup>37</sup> Acteoside significantly influenced osteoclastogenesis by down-regulated *NFKBIA* expression to prevent the development of osteoporosis.<sup>38</sup> Most of these genes have been linked to the treatment of mechanism of OP. However, *RXRG*, *RXR*, *THRB*, and



**Figure 11.** Molecular docked structure of IL1B-ginsenoside complex. Docking was performed using AutoDock software (V4.2.6) and various amino acid interactions are mentioned in the respective passage. The larger helical lamellar structure is IL1B protein and the smaller hexagon is ginsenoside.

*RARG* have not been demonstrated to be associated with OP or ginsenosides in the previous studies. This implicates that these genes might be new potential targets for ginsenosides treating OP worth being explored in the future.

### The Functional and pathway enrichment analysis

As shown in the Figure 4, the enrichment analysis built by String can provide a great number of enrichment results regarding to GO and KEGG. In the results of GO, steroid hormone is one of most important factor. It is “response to steroid hormone” in BP, “steroid hormone receptor activity” and “hormone receptor binding” in MF, and in CC. OP has been considered as one of most common adverse side effects after glucocorticoid therapy. Recent studies have suggested that the changes of steroid hormone levels are important factors in the development of OP.<sup>39</sup> Moreover, some researches have found that ginsenosides could attenuate the glucocorticoid-induced OP. Zhang et al. in their study reported that ginsenosides Rg3 weakened glucocorticoid-induced OP through regulating BMP-2/BMPRI1A/Runx2 signaling pathway.<sup>40</sup> In Gao et al.’s report, they described that ginsenoside-Rb2 promoted GPR120 induction in bone marrow-derived mesenchymal stem cells to inhibit dexamethasone-induced OP.<sup>10</sup> The results of the KEGG enrichment analysis showed that the cancer pathway were more enriched compared with the osteoclast differentiation signaling pathways. A possible explanation is that the cancer pathways are more associated with ginsenoside. Some examples have demonstrated that different ginsenosides active ingredients have anti-tumor effect in various cancers.<sup>41,42</sup> However,

ginsenosides and osteoclast differentiation also have a strong correlation. Ginsenosides prevent osteoporosis by inhibiting osteoclast differentiation. Ginsenoside Rb3 reduced osteoclast generation by inhibiting the MAPK/AKT/NF- $\kappa$ B signaling pathways.<sup>43</sup> Another report showed that ginsenoside Rb2 inhibits osteoclast differentiation associated with blocking NF- $\kappa$ B and STAT3 signaling pathways.<sup>44</sup> Our GO and KEGG results show ginsenosides may attenuate steroid hormone-induced OP by inhibiting osteoclast differentiation.

### Prediction of ginsenosides binding to target proteins

The results shown in the Figures 10 and 11 represent the detail 3D optimal docking images, which indicates that ginsenosides can bind with different targets and played a role in anti-osteoporotic. What the most valuable in these outcomes is that ginsenoside not only could combine with their targets to play a role, but also present the precise docking site of the targets. This computational method has been demonstrated to play a key role in the development of drug discovery/design. Furthermore, the advantage of this molecular docking is to reduce time and save money, lastly, accelerate the identification of potential drug candidate.<sup>45</sup> As we have known isothermal titration calorimetry, circular dichroism, nuclear magnetic resonance, atomic force microscope, and molecular docking method are the common approaches to study the interaction between proteins and small molecule. The Autodock software was used to verify the results of that from Swissdock. This provided believable and meaningful outcomes and could save the time in this kind of study.

### The value of our study

We apply the network pharmacology approach to illustrate the molecular mechanisms of ginsenosides treating OP in this study. The network pharmacology suggests great advantages in exploring the types of drugs and mode of action. Additionally, let me emphasize the limitations of this study. First, our research is based on pharmacological network methods to study potential targets of ginsenosides in treating OP, lacking cell and animal experiments to support. Second, because of the difficulty of obtaining data, the database might not contain all known or unknown key targets and protein–protein interactions. This could be improved in the future if more data becomes available.

### Conclusion

In the present study, 14 potential targets of ginsenosides treatment for OP were determined by a network pharmacological method, which was validated by functional



enrichment analysis. *IL1B*, *TNF*, *IFNG*, and *NFKBIA* were the most important target proteins. The mechanisms used by ginsenosides to treat OP are elucidated. Osteoclast differentiation is the most valuable signaling pathway. Our study laid a good theoretical foundation for further research of the ginsenosides treatment of OP.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This present study was supported by funds from the Key Technology Research and Development Program of Shandong (grant no. 2019GSF108257 and 2018GSF118192) and Shandong Provincial Medical and Health Science and Technology Development Project (2019WS500). Funding agencies have no role in research design or manuscript writing.

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