Research Article

Identified the Synergistic Mechanism of Drynariae Rhizoma for Treating Fracture Based on Network Pharmacology

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1. Introduction

Fracture is a common and frequent disease that occurs in patients with various injuries or osteoporosis [1]. In China, the population-weighted incidence of traumatic fractures of the legs, arms, or trunk in 2014 was 3.21 per 1,000 people (95% CI 2.83–3.59) [2]. Osteoporotic fractures are estimated to account for half of all fractures by 2050, and the estimated cost of osteoporotic hip fractures worldwide may reach $131 billion [3]. Therefore, the study of drugs for the prevention and treatment of fractures plays an important role in promoting patient health and reducing family economic pressure.

Recently, DR, one of the plants from Davalliaceae and Davallia Sm., has been widely used in the prevention and treatment of various fractures due to excellent treatment,
low side effects, extensive use, and safety [4]. Animal experiments have confirmed that DR could alter the bone histomorphology and increase the number of trabeculae by 10% [5], and its osteogenesis is related to Runx2 and BMP-2 signaling pathways [6]. In addition, it is believed that the various ingredients contained in an herb could regulate multiple targets in different signaling pathways and produce synergistic therapeutic effects [7]. However, such research has not been carried out in the treatment of fractures with DR.

Network pharmacology based on systems biology and polypharmacology has achieved a paradigm shift from “one drug, one goal” to “multi-ingredient therapy, biological network,” which has attracted the attention of Chinese medicine researchers and has been recognized as an effective tool for elucidating multiple components, targets, synergistic effects, and mechanisms of Chinese medicine [8–10]. It is reported that network pharmacology predicts the clinical efficacy, pathways, and side effects of drugs by constructing drug-drug networks, disease-drug networks, and disease-disease networks, providing valuable information for improving the clinical efficacy, reducing toxicity, and elucidating multimechanisms of drugs [11]. For example, Wang Nani found that Er-Xian Decotion has 13 main components closely related to 65 osteoporosis-related targets by using network pharmacology, thereby constructing Er-Xian Decotion component-osteoporosis target network and potential antosteoporosis mechanism [12]. Yueying et al. identified 108 compounds, 86 potential targets, and 47 signal transduction pathways that Danshiliubao Granule regulates liver fibrosis by the network pharmacology method, which reflects the multicomponent, multitarget, and multichannel characteristics of Chinese herbal medicine in antiliver fibrosis [13]. Therefore, in order to reveal the relationship between fracture and the active ingredients involved in the DR, we conducted network pharmacology to achieve this goal from protein and gene level. We collected the information of targets from active ingredients in DR and targets of fracture from several databases, respectively, and used network pharmacology to explore the potential synergistic mechanisms of DR for treating fracture.

2. Materials and Methods

2.1. Screening of Active Ingredients of Drynariae Rhizoma. Traditional Chinese Medicine Systems Pharmacology (TCMSP, http://lsp.nwu.edu.cn/, Version 2.3) Database and Analysis Platform includes chemicals, targets, and drug-target-disease networks, as well as pharmacokinetic properties involving oral bioavailability, druglikeness, blood-brain-barrier, and so on [14]. There were 71 compounds of DR which were obtained from the TCMSP. The potential active ingredients of DR for treating fracture were screened according to their oral bioavailability (OB) ≥30% and druglikeness (DL) ≥0.18 recommended by TCMSP.

2.2. Obtaining the Chemical Structure of Active Ingredients. The structure of the potential active ingredients of DR was downloaded from TCMSP and stored in mol2 format. If there was no chemical structure, the PubChem compound was put into the PubChem (https://pubchem.ncbi.nlm.nih.gov/) to download a chemical structure and save it in sdf format, or the PubChem compound was put into the Zinc database (https://zinc.docking.org/) to download a chemical structure and save it in mol2 format. The related SMILES of potential active ingredients was received from TCMSP or PubChem or Zinc database. Then, the SMILES was put into the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) to obtain the related drug target and save it.

2.3. Gene Targets of Drynariae Rhizoma. The DRAR-CPI server (http://cpi.bio-x.cn/drar, update in 2017-7-26) has a collection of drug molecules and targetable human proteins [15]. When submitting a drug molecule, the server docks the drug uploaded by users with the three-dimensional structure of all protein targets in the database, scores, and ranks them with the affinity scoring function based on the protein-ligand interaction, thereby predicting the potential protein targets of human-targetable drugs [15, 16]. This affinity score is called Z-score in the DRAR-CPI server [17]. Protein-ligand interaction with Z-score < −0.5 was recommended by DRAR-CPI as a potential protein target for human-targetable drugs [16]. We uploaded the potential active ingredients of DR in mol2 or sdf format and used Z-score < −0.5 to select potential protein targets for DR. A total of 1760 proteins with Z-score < −0.5 and 355 protein targets were obtained after deletion of the duplicate data. The PDB ID of the protein targets were inputted into UniProt KB (http://www.uniprot.org/uniprot/) of the UniProt database, and the “popular organisms” was selected as human to obtain the gene targets associated with the potential active ingredient of DR.

2.4. Gene Target Prediction for Drynariae Rhizoma to Treat Fractures. The following electronic databases were searched to identify the genes related to fractures: Genetic Association Database (https://geneticassociationdb.nih.gov/), Therapeutic Targets Database (http://bidd.nus.edu.sg/BIDD-Databases/TTD/TTD.asp), PharmGkb database (https://www.pharmgkb.org/), GeneCards database (http://www.genecards.org/), and OMIM database (http://www.ncbi.nlm.nih.gov/omim). Then, the duplicate data and false-positive genes were deleted. Finally, the Venny tool (http://bioinogg.cnbbi.cscie.es/tools/venny/index.html, Version 2.1) was used to identify the common gene targets of DR and fracture, which may be the potential targets for DR to treat fractures.

2.5. Constructing the Ingredient-Target Network of Drynariae Rhizoma. The common gene targets of DR and fracture were introduced into the Cytoscape software (Version 3.4.0) to construct an ingredient-target network of Drynariae Rhizoma and analyze the topology properties of the network, including degree, betweenness centrality, and closeness centrality [18]. The degree describes the number of connections to a node in the network, indicating interaction with other nodes in the network. Betweenness centrality
measures the proportion of a node between shortest paths among other nodes, suggesting the importance of nodes in maintaining network tightness. Closeness centrality indicates the degree of nodes close to the “center” of the network. A node with high degree, betweenness centrality, and closeness centrality values means that it plays a very important role in the network [18].

2.6. Constructing Protein-Protein Interaction (PPI) of Drynariae Rhizoma. The String database (https://string-db.org/, Version 10.5) is a database containing known and predicted PPIs, which collect and integrate a large number of protein interactions involving 9,643,763 proteins and 1,380,838,440 interactions, including experimental data and interactive prediction data derived from bioinformatic methods [19]. Common gene targets of DR and fracture were imported into the STRING database, and the species were set to humans for PPIs. Then, the highest confidence was set to 0.9 in the minimum required interaction score and the results were updated. The TSV format of the updated results were downloaded. Then, node1, node2, and combined scores were extracted and imported into the Cytoscape software to create a PPI network, and the network was analyzed as follows: Step 1: analyze the topological properties of the network: cytoscape → tools → network analyzer → network analysis → analyze network, save the CSV format of the network result and extract the degree value. Step 2: create a network map according to the degree: cytoscape → tool → network analyzer → network analysis → generate style from statistics → map node size to degree → map node color to degree and save the PPI network map.

2.7. Molecular Docking. SystemsDock (http://systemsdock.unit.oist.jp, Version 2.0) is a web server for network pharmacology-based prediction and analysis that could be used to illustrate the role of ligands on a complex molecular network [20]. It evaluates the protein-ligand binding potential of molecular docking by combining docking with the intelligence (dock-IN) score. The dock-IN score is the negative logarithm of the experimental dissociation/inhibition constant (pKD/pKi), which ranges from 0 to 10, indicating weak to strong binding [20]. It is believed that the dock-IN score above 4.25 indicates a slight binding potential between the protein and ligand; a value greater than 5.0 indicates a moderate binding potential, and a value greater than 7.0 indicates a strong binding potential [16]. We extracted the top 5 proteins with the highest degree value in the PPI network. The proteins that were recognized by systemsDock docked with the potential active ingredients of DR to receive the dock-IN score. The results were saved, and their dock-IN score was analyzed to assess the binding potentials between the potential active ingredients of DR and protein targets.

2.8. GO Functional Analysis and KEGG Pathway Enrichment Analysis. GO (http://www.geneontology.org) is widely used for annotation of gene function, providing detailed annotations of gene function in terms of biological process (BP), cellular component (CC), and molecular function (MF), respectively [21]. Database for annotation, visualization, and integrated discovery (David, https://david.ncifcrf.gov/, Version 6.8) is a functional genomic annotation database that provides bioinformatics annotation for genes or proteins based on the gene annotation function of the GO database and the signaling pathway information of the KEGG database [22]. We performed GO functional analysis and KEGG pathway enrichment analysis in the David database. The procedure was as follows: Step 1: paste the common gene targets of DR and fracture list. Step 2: select “OFFICIAL_GENE_SYMBOL” in “Select Identifier.” Step 3: select “Gene List” in “List Type.” Step 4: select “Homo sapiens” in species. Step 5: submit list. Step 6: download the results of BP, CC, and MF in the gene ontology. Step 7: download the results of KEGG pathway in the pathways. Step 8: targets with $P < 0.05$ were screened and sorted by count (number of targets), and the top-ranked biological processes or KEGG pathways were extracted. Step 9: BP, CC, and MF were designed using GraphPad Prism 5.0 software. The KEGG pathways were designed by the advanced bubble chart of the omicshare (http://omicshare.com/tools/Home/Soft/getsoft/type/index).

2.9. Collect Protein Class Corresponding to Common Gene Targets. DisGeNET (http://www.disgenet.org/web/DisGeNET/menu, Version 5.0) is a discovery platform that contains one of the largest publicly available genes and variants associated with human disease. It could be used to analyze the properties of disease genes and investigate the molecular basis of specific diseases and their comorbidities, as well as adverse drug reactions [23]. We used the search function of the DisGeNET platform to retrieve the protein class corresponding to common gene targets.

2.10. Pathway Integration. We used the KEGG Mapper tool in the KEGG database (http://www.kegg.jp/) to retrieve some pathways of DR for fractures and then integrate into a final pathway map. The procedure was as follows: Step 1: used the UniProt KB search function of the UniProt database to retrieve the UniprotID of the common gene targets. Step 2: import the UniProt ID of the common gene targets. Step 3: set the parameters: search against: hsa, primary ID: NCBI-UniProt ID, and examples: Homo sapiens pathway. Step 4: download the PI3K-AKT, MAPK, Ras, and VEGF signaling pathways. Step 5: integrate the signal path.

3. Results

3.1. Active Ingredients of Drynariae Rhizoma. A total of 71 ingredients of DR were retrieved from TCMSP, and 18 active ingredients were screened according to the biological functions of DR. However, marioside_qt (Molecule ID: MOL009087) was removed because it could not be recognized by the PubChem or Zinc database. The remaining 17 active ingredients are shown in Table 1, including (2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one, auresudin, eriodictyol (flavanone), stigmasterol, beta-
| No. | Molecule ID   | Molecule name             | Chemical formula | Structure | OB (%) | DL  |
|-----|---------------|---------------------------|------------------|-----------|--------|-----|
| 1   | MOL001040     | (2R)-5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one | C_{15}H_{12}O_{5} | ![Structure](structure1.png) | 42.36  | 0.21 |
| 2   | MOL001978     | Aureusidin                | C_{13}H_{10}O_{6} | ![Structure](structure2.png) | 53.42  | 0.24 |
| 3   | MOL002914     | Eriodictyol (flavanone)   | C_{15}H_{12}O_{6} | ![Structure](structure3.png) | 41.35  | 0.24 |
| 4   | MOL000449     | Stigmasterol              | C_{29}H_{48}O     | ![Structure](structure4.png) | 43.83  | 0.76 |
| 5   | MOL000358     | β-Sitosterol               | C_{29}H_{50}O     | ![Structure](structure5.png) | 36.91  | 0.75 |
| 6   | MOL000422     | Kaempferol                | C_{15}H_{10}O_{6} | ![Structure](structure6.png) | 41.88  | 0.24 |
| No. | Molecule ID   | Molecule name     | Chemical formula | Structure | OB (%) | DL  |
|-----|---------------|-------------------|------------------|-----------|--------|-----|
| 7   | MOL004328     | Naringenin        | C_{12}H_{12}O_{5} | ![Structure](image) | 59.29  | 0.21 |
| 8   | MOL000492     | (+)-Catechin      | C_{15}H_{14}O_{6} | ![Structure](image) | 54.83  | 0.24 |
| 9   | MOL005190     | Eriodictyol       | C_{15}H_{12}O_{6} | ![Structure](image) | 71.79  | 0.24 |
| 10  | MOL000569     | Digallate         | C_{14}H_{10}O_{9} | ![Structure](image) | 61.85  | 0.26 |
| 11  | MOL000006     | Luteolin          | C_{15}H_{10}O_{6} | ![Structure](image) | 36.16  | 0.25 |
| 12  | MOL009061     | 22-Stigmasten-3-one | C_{29}H_{48}O | ![Structure](image) | 39.25  | 0.76 |
sitosterol, kaempferol, naringenin, (+)-catechin, eriodictyol, digallate, luteolin, 22-stigmasten-3-one, cyclolaudenol acetate, cycloartenone, cyclolaudenol, davallioside A_qt, and xanthogalenol.

3.2. Gene Target Prediction. A total of 303 gene targets associated with the potential active ingredients of DR were retrieved in the UniProt database. A total of 3,173 fracture-related genes were received, and 3,054 genes remained after deletion of the duplicate and false-positive genes. Common gene target screening for fracture and DR are shown in Figure 1. A total of 144 common gene targets of DR and fracture were received, indicating the potential targets for DR to treat fractures, as shown in Table 2.

| No. | Molecule ID   | Molecule name          | Chemical formula | Structure | OB (%) | DL |
|-----|---------------|------------------------|------------------|-----------|--------|----|
| 13  | MOL009063     | Cyclolaudenol acetate  | C_{33}H_{54}O_{2} | ![Structure](structure1.png) | 41.66  | 0.79 |
| 14  | MOL009075     | Cycloartenone          | C_{30}H_{48}O    | ![Structure](structure2.png) | 40.57  | 0.79 |
| 15  | MOL009076     | Cyclolaudenol          | C_{31}H_{52}O    | ![Structure](structure3.png) | 39.05  | 0.79 |
| 16  | MOL009078     | Davallioside A_qt      | C_{25}H_{29}NO_{12} | ![Structure](structure4.png) | 62.65  | 0.51 |
| 17  | MOL009091     | Xanthogalenol          | C_{21}H_{22}O_{5} | ![Structure](structure5.png) | 41.08  | 0.32 |

Figure 1: Venn diagram of common gene target screening for fracture and *Drynariae Rhizoma*. Table 1: Continued.
Table 2: Information of potential gene targets for treating fracture from *Drynariae Rhizoma*.

| No. | PDB ID | Gene target |
|-----|--------|-------------|
| 1   | 1HSZ   | ADH1B       |
| 2   | 1HT0   | ADH1C       |
| 3   | 1D1T   | ADH7        |
| 4   | 1H0C   | AGXT        |
| 5   | 3CQW   | AKT1        |
| 6   | 1O6L   | AKT2        |
| 7   | 2GLQ   | ALPP        |
| 8   | 1ANG   | ANG         |
| 9   | 1HAK   | ANXA5       |
| 10  | 1E3G   | AR          |
| 11  | 2NZ2   | ASS1        |
| 12  | 1ONQ   | B2M         |
| 13  | 1XLV   | BCHE        |
| 14  | 1ES7   | BMP2        |
| 15  | 1M4U   | BMP7        |
| 16  | 1ES7   | BMPR1A      |
| 17  | 1UWJ   | BRAF        |
| 18  | 1A42   | CA2         |
| 19  | 1HCE   | CASP1       |
| 20  | 1K86   | CASP7       |
| 21  | 2C2Z   | CASP8       |
| 22  | 2HRB   | CBR3        |
| 23  | 1JBQ   | CBS         |
| 24  | 1ONQ   | CD1A        |
| 25  | 1POZ   | CD44        |
| 26  | 2OBD   | CETF        |
| 27  | 1XMI   | CFRTR       |
| 28  | 3DRB   | CKB         |
| 29  | 1NN6   | CMA1        |
| 30  | 3BWy   | COMT        |
| 31  | 1NM8   | CRAT        |
| 32  | 1C8P   | CSF2RB      |
| 33  | 1BYG   | CSK         |
| 34  | 1CSB   | CTSB        |
| 35  | 1LYW   | CTSD        |
| 36  | 1CGH   | CTSG        |
| 37  | 1JKL   | DAPK1       |
| 38  | 2HHA   | DPP4        |
| 39  | 1M17   | EGFR        |
| 40  | 1H1B   | ELANE       |
| 41  | 1R5K   | ESR1        |
| 42  | 1QKM   | ESR2        |
| 43  | 2PjL   | ESRR1       |
| 44  | 1F0R   | F10         |
| 45  | 1A3B   | F2          |
| 46  | 1Z6J   | F3          |
| 47  | 1Z6J   | F7          |
| 48  | 1RFN   | F9          |
| 49  | 2FGI   | FGFR1       |
| 50  | 2Pvy   | FGFR2       |
| 51  | 2BH9   | G6PD        |
| 52  | 1ZNOQ  | GAPDH       |
| 53  | 1OgS   | GBA         |
| 54  | 1J78   | GC          |
| 55  | 1PUb   | GM2A        |
| 56  | 1j1B   | GSK3B       |
| 57  | 1GRE   | GSR         |
| 58  | 1XWK   | GSTM1       |
| 59  | 1IGS   | GSTP1       |

Table 2: Continued.

| No. | PDB ID | Gene target |
|-----|--------|-------------|
| 60  | 2C3Q   | GST1        |
| 61  | 2VQM   | HDAC4       |
| 62  | 1GMN   | HGF         |
| 63  | 1H WL  | HMGC        |
| 64  | 1S8C   | HMOX1       |
| 65  | 5P21   | HRAS        |
| 66  | 1DHT   | HSD17B1     |
| 67  | 1ZBQ   | HSD17B4     |
| 68  | 1YET   | HSP90A1A1   |
| 69  | 2OJ9   | IGF1R       |
| 70  | 1ZT3   | IGBP1       |
| 71  | 2ILK   | IL10        |
| 72  | 1G0Y   | IL1R1       |
| 73  | 2CYK   | IL4         |
| 74  | 1TYL   | INS         |
| 75  | 2AUH   | INSR        |
| 76  | 1QC Y  | ITGA1       |
| 77  | 2B7A   | JAK2        |
| 78  | 1ZSX   | KCNA2       |
| 79  | 1QPC   | LCK         |
| 80  | 1IT0   | LDHB        |
| 81  | 1KJL   | LGALS3      |
| 82  | 1TVO   | MAPK1       |
| 83  | 1JNK   | MAPK10      |
| 84  | 1A9U   | MAPK14      |
| 85  | 1UK1   | MAPK8       |
| 86  | 2DFD   | MDH2        |
| 87  | 1GCZ   | MIF         |
| 88  | 1DMT   | MME         |
| 89  | 1HFC   | MMP1        |
| 90  | 1QIA   | MMP3        |
| 91  | 1JAP   | MMP8        |
| 92  | 1SD2   | MTAP        |
| 93  | 2P54   | NCOA1       |
| 94  | 1MVC   | NCOA2       |
| 95  | 2IIP   | NNMT        |
| 96  | 1M4U   | NOG         |
| 97  | 1INS   | NOS2        |
| 98  | 1KBO   | NQ01        |
| 99  | 1UPV   | NR1H2       |
| 100 | 3FXV   | NR1H4       |
| 101 | 1NRL   | NR1H2       |
| 102 | 1P93   | NR3C1       |
| 103 | 2A3J   | NR3C2       |
| 104 | 1YOW   | NR5A1       |
| 105 | 1WWA   | NTRK1       |
| 106 | 1WWB   | NTRK2       |
| 107 | 1OTH   | OTC         |
| 108 | 1WOK   | PARP1       |
| 109 | 2YQK   | PDE4A       |
| 110 | 1PTW   | PDE4D       |
| 111 | 1ZUC   | PGR         |
| 112 | 2VGB   | PKLR        |
| 113 | 1VJA   | PLAU        |
| 114 | 2PK4   | PLG         |
| 115 | 1NRG   | PNPO        |
| 116 | 1V04   | PON1        |
| 117 | 1BI C  | POR         |
| 118 | 2P54   | PPARA       |
| 119 | 2J14   | PPARD       |
Degree in the network indicates the number of proteins that a protein has interacting with. In other words, top-degree protein targets screened in PPI plays a pivotal role in the treatment of fractures with DR. Five important protein targets with top degree of DR were identified in the PPI network and are shown in Table 4. They were MAPK1, SRC, HRAS, RXRA, and NCOA1.

3.5. Molecular Docking. Three important protein targets with top degree of DR were identified by SystemsDock, including SRC, RXRA, and NCOA1. Dock-IN score of these three proteins docked with 17 active ingredients of DR are shown in Table 5. Molecular docking results showed that there were 17 (33.33%) with a dock-IN score greater than 7.0, 24 (47.06%) with a dock-IN score between 7.0 and 5.0, 8 (15.69%) with a dock-IN score between 5.0 and 4.25, and 2 (3.92%) with a dock-IN score less than 4.25.

3.6. Gene Ontology (GO) Functional Analysis and KEGG Pathway Enrichment Analysis. Enriched gene ontology terms for BP, CC, and MF of potential therapeutic fracture targets from the main active ingredients of DR are shown in Figure 4. In the BP (Figure 4(a)), positive regulation of transcription from RNA polymerase II promoter involved 33 (22.92%) potential therapeutic fracture targets, signal transduction involved 30 (20.84%) potential therapeutic fracture targets, negative regulation of transcription from RNA polymerase II promoter involved 20 (13.89%) potential therapeutic fracture targets, positive regulation of transcription and DNA-template involved 19 (13.19%) potential therapeutic fracture targets, and transcription initiation from RNA polymerase II promoter involved 18 (12.5%) potential therapeutic fracture targets. In the CC (Figure 4(b)), cytosol involved 63 (43.75%) potential therapeutic fracture targets, extracellular exosome involved 60 (41.67%) potential therapeutic fracture targets, cytoplasm involved 58 (40.28%) potential therapeutic fracture targets, nucleus involved 56 (38.89%) potential therapeutic fracture targets, and plasma membrane involved 53 (36.81%) potential therapeutic fracture targets. In the MF (Figure 4(c)), protein binding involved 110 (76.39%) potential therapeutic fracture targets, zinc ion binding involved 31 (21.53%) potential therapeutic fracture targets, identical protein binding involved 29 (20.14%) potential therapeutic fracture targets, ATP binding involved 27 (18.75%) potential therapeutic fracture targets, and enzyme binding involved 23 (15.97%) potential therapeutic fracture targets.

Enriched KEGG pathways of potential targets for treating fracture from the main active ingredients of DR are shown in Figure 5. The MAPK signaling pathway was identified as an important signaling pathway involving 17 (11.81%) potential therapeutic fracture targets with $P = 7.82 \times 10^{-6}$. The PI3K-Akt signaling pathway involved 17 (11.81%) potential therapeutic fracture targets, the Rap1 signaling pathway involved 14 (9.72%) potential therapeutic fracture targets, the Ras signaling pathway...
involved 14 (9.72%) potential therapeutic fracture targets, and the signaling pathways regulating pluripotency of stem cells involved 12 (9.03%) potential therapeutic fracture targets.

3.7. Protein Class Corresponding to Common Gene Targets. The protein class corresponding to potential targets for treating fracture from the main active ingredients of DR is presented in Table 6. The results showed that DR treatment

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Table 3: Gene targets with the top 4 degree values, betweenness centrality, and closeness centrality.

| No. | Gene targets | Degree (rank) | Betweenness centrality (rank) | Closeness centrality (rank) |
|-----|--------------|---------------|-------------------------------|----------------------------|
| 1   | NCOA1        | 15 (1)        | 0.038 (1)                     | 0.505 (1)                  |
| 2   | MAPK1        | 13 (2)        | 0.023 (3)                     | 0.464 (4)                  |
| 3   | GSK3B        | 12 (3)        | 0.028 (2)                     | 0.502 (2)                  |
| 4   | TTPA         | 12 (4)        | 0.022 (4)                     | 0.466 (3)                  |
of the fracture process involved a variety of substances, such as signaling molecule, transcription factor, receptor, enzyme modulator, chaperone, cell adhesion molecule, protein (transporter, transfer protein, carrier protein, calcium-binding protein, defense protein, and immune protein), enzyme modulator, and enzymes (oxidoreductase, kinase, phosphatase, hydrolase, ligase, protease, isomerase, lyase, enzyme regulator, and transferase).

3.8. Signaling Pathway Integration. Four pathways associated with the potential targets of DR main active ingredients for treating fracture are presented in Figure 6.

![Figure 3: Protein-protein interaction network of Drynariae Rhizoma. Note. The size and the color of the node represents the value of the degree (yellow ➔ orange ➔ blue indicates that the degree value is from low to high, and the small circle represents a low degree value).](image)

Table 4: Five important protein targets with top degree of Drynariae Rhizoma.

| No. | Degree | PDB ID | Protein target name |
|-----|--------|--------|---------------------|
| 1   | 27     | 1TVO   | MAPK1               |
| 2   | 23     | 1YOL   | SRC                 |
| 3   | 22     | 5P2I   | HRAS                |
| 4   | 20     | 1MVC   | RXRA                |
| 5   | 18     | 2P54   | NCOA1               |

The arrow (➔) indicates the promoting effect, the T-arrows (+) indicate the inhibition, and the arrows of different colors represent different signaling pathways. The targets of the signaling pathway were marked as light blue, and the potential targets of DR main active ingredients for treating fracture were marked as dark blue. There were 21 (14.58%) potential targets of main active ingredients of DR for treating fracture in the PI3K-AKT, MAPK, Ras, and VEGF signaling pathways, indicating that the fracture targets play a role in these signaling pathways. In addition, some targets play a role in a variety of signaling pathways, such as Ras, RafB, AKT/PKB, PI3K, ERK, and JNK.

4. Discussion

In order to reveal the relationship between fracture and the active ingredients involved in the DR, we predicted the mechanism of DR treatment fractures by constructing a biological network of interactions between active ingredients and common gene targets and common protein targets from a molecular level. A total of 17 active ingredients of DR were received in our study, including (2R)-5,7-dihydroxy-2-(4-
hydroxyphenyl)chroman-4-one, aureusidin, eriodictyol (flavanone), stigmasterol, beta-sitosterol, naringenin, (+)-catechin, eriodictyol, digallate, luteolin, 22-stigmasten-3-one, cyclolaudenol acetate, cycloartenone, cyclolaudenol, davallioside A_qt, and xanthogalenol. Most of them were polyphenolic compounds, which are also called flavonoids. Flavonoids are considered to be the main active ingredients of DR and have been reported to reduce bone loss in ovariectomized rats [24]. In addition, Kang Suk-Nam finds that the total phenolics and flavonoids of DR are better.

Table 5: Molecular docking of three important protein targets from *Drynariae Rhizoma*.

| Protein target | PDB ID  | Ingredients                                      | Dock-IN score |
|----------------|---------|--------------------------------------------------|---------------|
| NCOA1          | 1NQ7    | (+)-Catechin                                     | 7.111         |
| RXRA           | 1DSZ    | (+)-Catechin                                     | 4.624         |
| SRC            | 1O4R    | (+)-Catechin                                     | 5.908         |
| NCOA1          | 1NQ7    | (2R)-5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one | 6.694         |
| RXRA           | 1DSZ    | (2R)-5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one | 4.605         |
| SRC            | 1O4R    | (2R)-5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one | 5.783         |
| NCOA1          | 1NQ7    | 22-Stigmasten-3-one                              | 8.422         |
| RXRA           | 1DSZ    | 22-Stigmasten-3-one                              | 5.533         |
| SRC            | 1O4R    | 22-Stigmasten-3-one                              | 5.425         |
| NCOA1          | 1NQ7    | Aureusidin                                       | 7.153         |
| RXRA           | 1DSZ    | Aureusidin                                       | 4.622         |
| SRC            | 1O4R    | Aureusidin                                       | 5.861         |
| NCOA1          | 1NQ7    | Beta-sitosterol                                   | 8.34          |
| RXRA           | 1DSZ    | Beta-sitosterol                                   | 5.878         |
| SRC            | 1O4R    | Beta-sitosterol                                   | 5.374         |
| NCOA1          | 1NQ7    | Cycloartenone                                    | 8.427         |
| RXRA           | 1DSZ    | Cycloartenone                                    | 5.658         |
| SRC            | 1O4R    | Cycloartenone                                    | 5.693         |
| NCOA1          | 1NQ7    | Cyclolaudenol                                    | 8.376         |
| RXRA           | 1DSZ    | Cyclolaudenol                                    | 5.534         |
| SRC            | 1O4R    | Cyclolaudenol                                    | 5.376         |
| NCOA1          | 1NQ7    | Cyclolaudenol acetate                            | 8.422         |
| RXRA           | 1DSZ    | Cyclolaudenol acetate                            | 7.052         |
| SRC            | 1O4R    | Cyclolaudenol acetate                            | 6.364         |
| NCOA1          | 1NQ7    | Davallioside A_qt                                | 7.904         |
| RXRA           | 1DSZ    | Davallioside A_qt                                | 5.779         |
| SRC            | 1O4R    | Davallioside A_qt                                | 5.58          |
| NCOA1          | 1NQ7    | Digallate                                        | 4.313         |
| RXRA           | 1DSZ    | Digallate                                        | 3.789         |
| SRC            | 1O4R    | Digallate                                        | 3.671         |
| NCOA1          | 1NQ7    | Eriodictyol                                      | 7.109         |
| RXRA           | 1DSZ    | Eriodictyol                                      | 4.628         |
| SRC            | 1O4R    | Eriodictyol                                      | 5.821         |
| NCOA1          | 1NQ7    | Eriodictyol (flavanone)                          | 7.113         |
| RXRA           | 1DSZ    | Eriodictyol (flavanone)                          | 4.633         |
| SRC            | 1O4R    | Eriodictyol (flavanone)                          | 5.824         |
| NCOA1          | 1NQ7    | Kaempferol                                       | 7.125         |
| RXRA           | 1DSZ    | Kaempferol                                       | 4.613         |
| SRC            | 1O4R    | Kaempferol                                       | 5.929         |
| NCOA1          | 1NQ7    | Luteolin                                         | 7.089         |
| RXRA           | 1DSZ    | Luteolin                                         | 4.621         |
| SRC            | 1O4R    | Luteolin                                         | 5.847         |
| NCOA1          | 1NQ7    | Naringenin                                       | 7.12          |
| RXRA           | 1DSZ    | Naringenin                                       | 6.016         |
| SRC            | 1O4R    | Naringenin                                       | 5.883         |
| NCOA1          | 1NQ7    | Stigmasterol                                     | 8.376         |
| RXRA           | 1DSZ    | Stigmasterol                                     | 5.918         |
| SRC            | 1O4R    | Stigmasterol                                     | 5.3           |
| NCOA1          | 1NQ7    | Xanthogalenol                                    | 5.387         |
| RXRA           | 1DSZ    | Xanthogalenol                                    | 7.093         |
| SRC            | 1O4R    | Xanthogalenol                                    | 7.142         |
extracted with 70% ethanol instead of water, and this ethanol extraction method also makes these extracts have higher antioxidant activity and in vitro antiosteoporosis effect [25]. In the ingredient-target network, all active ingredients were also identified to bind well to the fracture gene targets, binding to at least 29 (20.14%) different gene targets. Therefore, the 17 active ingredients of DR may have the effect of reducing bone loss and promoting fracture healing.

In our study, 144 common gene targets of DR and fracture were received, and 774 interactions between the active ingredients of DR and common gene targets were found. Some gene targets have been confirmed by clinical trials or animal experiments. For example, Guimarães et al. found that polymorphisms in the FGFR1 and BMP4 genes were associated with fracture nonunion in patients [26]. And our team’s previous study also found that the total flavonoids of DP could promote osteogenesis and mineralization in rats with tibial defects by increasing the gene expression of BMP2, BMP4, BMPR1A, and Smad1 [27]. In the ingredient-target network, NCOA1, GSK3B, TTPA, and MAPK1 were identified as important gene targets based on degree values, betweenness centrality, and closeness centrality. Qin et al. found
Figure 5: Enriched KEGG pathways of potential targets for treating fracture from main active ingredients of *Drynariae Rhizoma*.

Table 6: The protein class corresponding to potential targets for treating fracture from main active ingredients of *Drynariae Rhizoma*.

| No. | Gene name | Protein class |
|-----|-----------|---------------|
| 1   | ADH1B     | Oxidoreductase |
| 2   | ADH1C     | Oxidoreductase |
| 3   | ADH7      | Oxidoreductase |
| 4   | AGXT      | Transferase    |
| 5   | AKT1      | Calcium-binding protein; kinase; transfer/carrier; protein; transferase |
| 6   | AKT2      | Calcium-binding protein; kinase; transfer/carrier; protein; transferase |
| 7   | ALPP      | Hydrolase; phosphatase |
| 8   | ANG       | None          |
| 9   | ANXA5     | None          |
| 10  | AR        | Nucleic acid binding; receptor; transcription factor |
| 11  | ASS1      | Ligase        |
| 12  | B2M       | Defense/immunity protein |
| No. | Gene name | Protein class                                    |
|-----|-----------|-------------------------------------------------|
| 13  | BCHE      | None                                            |
| 14  | BMP2      | Signaling molecule                              |
| 15  | BMP7      | Signaling molecule                              |
| 16  | BMPR1A    | Kinase; receptor; transferase                    |
| 17  | BRAF      | None                                            |
| 18  | CA2       | None                                            |
| 19  | CASP1     | Enzyme modulator; hydrolase; protease           |
| 20  | CASP7     | Enzyme modulator; hydrolase; protease           |
| 21  | CASP8     | Enzyme modulator; hydrolase; protease           |
| 22  | CBR3      | None                                            |
| 23  | CBS       | Hydrolase; isomerase; lyase                     |
| 24  | CD1A      | None                                            |
| 25  | CD44      | None                                            |
| 26  | CETP      | None                                            |
| 27  | CFTR      | Transporter                                      |
| 28  | CKB       | Kinase; transferase                              |
| 29  | CMA1      | Hydrolase; protease                              |
| 30  | COMT      | Transferase                                      |
| 31  | CRAT      | Transferase                                      |
| 32  | CSF2RB    | Receptor                                         |
| 33  | CSK       | None                                            |
| 34  | CTSB      | Enzyme modulator; hydrolase; protease           |
| 35  | CTSD      | Hydrolase; protease                              |
| 36  | CTSG      | Hydrolase; protease                              |
| 37  | DAPK1     | Kinase; transferase                              |
| 38  | DPP4      | Enzyme modulator; hydrolase; protease           |
| 39  | EGFR      | None                                            |
| 40  | ELANE     | Hydrolase; protease                              |
| 41  | ESR1      | Nucleic acid binding; receptor; transcription factor |
| 42  | ESR2      | Nucleic acid binding; receptor; transcription factor |
| 43  | ESRRB     | Nucleic acid binding; receptor; transcription factor |
| 44  | F10       | Hydrolase; protease                              |
| 45  | F2        | Hydrolase; protease                              |
| 46  | F3        | Defense/immunity protein; receptor              |
| 47  | F7        | Hydrolase; protease                              |
| 48  | F9        | Hydrolase; protease                              |
| 49  | FGFR1     | None                                            |
| 50  | FGFR2     | None                                            |
| 51  | G6PD      | Oxidoreductase                                   |
| 52  | GAPDH     | Oxidoreductase                                   |
| 53  | GBA       | None                                            |
| 54  | GC        | Transfer/carrier protein                        |
| 55  | GM2A      | Transfer/carrier protein                        |
| 56  | GSK3B     | Kinase; transferase                              |
| 57  | GSR       | Oxidoreductase                                   |
| 58  | GSTM1     | None                                            |
| 59  | GSTP1     | None                                            |
| 60  | GSTT1     | None                                            |
| 61  | HDAC4     | None                                            |
| 62  | HGF       | Hydrolase; protease                              |
| 63  | HMGCR     | None                                            |
| 64  | HMOX1     | Oxidoreductase                                   |
| 65  | HRAS      | Enzyme modulator                                |
| 66  | HSD17B1   | Oxidoreductase                                   |
| 67  | HSD17B4   | None                                            |
| 68  | HSP90AA1  | Chaperone                                        |
| 69  | IGF1R     | None                                            |
| 70  | IGFBP1    | Enzyme modulator                                |
| 71  | IL10      | None                                            |
| 72  | IL1R1     | Receptor                                         |
| No. | Gene name | Protein class                      |
|-----|-----------|------------------------------------|
| 73  | IL4       | None                               |
| 74  | INS       | None                               |
| 75  | INSR      | None                               |
| 76  | ITGA1     | None                               |
| 77  | JAK2      | None                               |
| 78  | KCNAB2    | Oxidoreductase; transporter         |
| 79  | LCK       | None                               |
| 80  | LDHB      | Oxidoreductase                     |
| 81  | LGALS3    | Cell adhesion molecule; signaling molecule |
| 82  | MAPK1     | Kinase; transferase                |
| 83  | MAPK10    | Kinase; transferase                |
| 84  | MAPK14    | Kinase; transferase                |
| 85  | MAPK8     | Kinase; transferase                |
| 86  | MDH2      | Oxidoreductase                     |
| 87  | MIF       | None                               |
| 88  | MME       | Hydrolase; protease                |
| 89  | MMP1      | Hydrolase; protease                |
| 90  | MMP3      | Hydrolase; protease                |
| 91  | MMP8      | Hydrolase; protease                |
| 92  | MTAP      | Transferase                        |
| 93  | NCOA1     | Transcription factor; transferase   |
| 94  | NCOA2     | Transcription factor; transferase   |
| 95  | NNMT      | Transferase                        |
| 96  | NOG       | None                               |
| 97  | NOS2      | None                               |
| 98  | NQO1      | None                               |
| 99  | NR1H2     | Nucleic acid binding; receptor; transcription factor |
| 100 | NR1H4     | Nucleic acid binding; receptor; transcription factor |
| 101 | NR1I2     | Nucleic acid binding; receptor; transcription factor |
| 102 | NR3C1     | Nucleic acid binding; receptor; transcription factor |
| 103 | NR3C2     | Nucleic acid binding; receptor; transcription factor |
| 104 | NR5A1     | Nucleic acid binding; receptor; transcription factor |
| 105 | NTRK1     | None                               |
| 106 | NTRK2     | None                               |
| 107 | OTC       | None                               |
| 108 | PARP1     | None                               |
| 109 | PDE4A     | None                               |
| 110 | PDE4D     | None                               |
| 111 | PGR       | Nucleic acid binding; receptor; transcription factor |
| 112 | PKLR      | None                               |
| 113 | PLAU      | Hydrolase; protease                |
| 114 | PLG       | Hydrolase; protease                |
| 115 | PNPO      | Oxidoreductase                     |
| 116 | PON1      | None                               |
| 117 | POR       | None                               |
| 118 | PPARA     | Nucleic acid binding; receptor; transcription factor |
| 119 | PPARD     | Nucleic acid binding; receptor; transcription factor |
| 120 | PPARG     | Nucleic acid binding; receptor; transcription factor |
| 121 | PPIB      | None                               |
| 122 | PRDX2     | Oxidoreductase                     |
| 123 | PRKACA    | None                               |
| 124 | PROCPR    | Enzyme modulator; receptor         |
| 125 | QDPR      | Oxidoreductase                     |
| 126 | RBP4      | Transfer/carrier protein           |
| 127 | REN       | Hydrolase; protease                |
| 128 | RXRA      | Nucleic acid binding; receptor; transcription factor |
| 129 | SEC14L2   | None                               |
| 130 | SHBG      | None                               |
| 131 | SLC9A3R1  | None                               |
| 132 | SOD1      | Oxidoreductase                     |
that NCOA1 promotes angiogenesis by upregulating HIF1α- and AP-1-mediated VEGFa transcription [28]. Galli et al. demonstrated by cell experiments that inhibition of GSK3B could increase cytoplasmic availability of b-catenin, thereby enhancing Wnt classical signaling and osteoblastic differentiation [29]. Fujita et al. found that mice deficient in TTPA developed a high bone mass phenotype in vertebrae and long bones due to lower bone resorption [30]. Matsushita et al. confirmed that MAPK1 (also called ERK2) plays an important role in osteoblast differentiation and osteoclastogenesis [31]. These gene targets are involved in vascularization, osteoblast differentiation, and osteoclastogenesis in fracture repair. Besides, we found that one active ingredient can interact with different gene targets, and one gene target can interact with different active ingredients, which is consistent with the modern drug theory of “multi-ingredient, multitarget” [9].

To identify the interactions of proteins corresponding to common genes, we conducted a PPI network. A total of 143 active ingredients were screened to identify potential targets. The results are shown in Table 6.

### Table 6: Continued.

| No. | Gene name | Protein class |
|-----|-----------|---------------|
| 133 | SRC       | None          |
| 134 | STS       | Hydrolase     |
| 135 | SULT2A1   | None          |
| 136 | THRA      | Nucleic acid binding; receptor; transcription factor |
| 137 | THRB      | Nucleic acid binding; receptor; transcription factor |
| 138 | TNF       | Signaling molecule |
| 139 | TPI1      | Isomerase     |
| 140 | TRAF2     | Signaling molecule |
| 141 | TTPA      | Transfer/carrier protein |
| 142 | TTR       | Transfer/carrier protein; transporter |
| 143 | TYMP      | Transferase   |
| 144 | VDR       | Nucleic acid binding; receptor; transcription factor |

**Figure 6:** Antifracture pathways of potential targets for treating fracture from main active ingredients of *Drynariae Rhizoma*. Note. The arrow (⟶) indicates the promoting effect, the T-arrows (⊣) indicate the inhibition, and the arrows of different colors represent different signaling pathways. The targets of the signaling pathway were marked as light blue, and the potential targets of main active ingredients of DR for treating fracture were marked as dark blue.
common protein targets for DR and fracture were received, with 315 PPIs. In addition, MAPK1, SRC, HRAS, RXRA, and NCOA1 were identified as the five most important target proteins. Previous studies have found that MAPK1 and SRC could promote proliferation and differentiation of myeloid cells and inhibit apoptosis [32, 33]. Clinical cases have found that elevated levels of fibroblast growth factor 23 in patients with dysplasia are associated with HRAS mutations [34]. RXRA is an essential cofactor in the action of 1,25-dihydroxyvitamin D, and umbilical cord RXRA methylation was inversely related to offspring bone mineral content [35]. Coronnello et al. found that NCOA1 modulate the estrogen effects in bone, and miR-488-5p overexpression reduces NCOA1 protein levels, thereby reducing bone mineral density [36]. These protein targets are associated with bone growth and angiogenesis in fracture repair. At the same time, we docked SRC, RXRA, and NCOA1 with 17 potential active ingredients of DR and found that 41 (80.39%) had moderate binding potential, suggesting that DR could bind well to fracture-related protein targets.

In order to identify the function of the common gene, we performed GO functional analysis on these genes. The results showed that the common gene involves multiple processes, parts and functions in BP, CC, and MF, which was consistent with existing studies about DR and fracture repair. For example, in the BP, 33 (22.92%) gene targets were involved in positive regulation of transcription from the RNA polymerase II promoter, and 30 (20.84%) gene targets were involved in signal transduction. Previous studies have shown that the promoter activates the polymerase to bind precisely to the template DNA and has the specificity of transcription initiation [37]. The RNA polymerase II promoter responsible for mRNA transcription is the largest and most important class of promoters [37]. This provides conditions for DR to initiate osteogenic targets. Besides, some signal transduction genes have been found in experiments. Song Nan found that VEGFR-2 may play a signal transduction role for naringin, one ingredient of DR, to stimulate angiogenesis and promote fracture healing [38]. In the CC, 63 (43.75%) gene targets were involved in cytosol, 60 (41.67%) gene targets were involved in extracellular exosome, and 58 (40.28%) gene targets were involved in cytoplasm. This indicates that the recovery of the fracture requires the support of various components in the cell, which is consistent with previous studies [39]. In the MF, 110 (76.39%) gene targets were involved in protein binding, suggesting that mutual recognition between proteins has good gene regulation conditions. This is consistent with the protein class corresponding to the potential target. These results were further validated in the protein class corresponding to the common gene. In the protein class, all of these common genes have been found to regulate a variety of fracture-related molecules, such as transcription factors, receptors, enzyme regulators, molecular chaperones, cell adhesion molecules, enzyme, and so on.

In order to identify the synergistic mechanism of DR for fracture, we performed KEGG pathway enrichment analysis and summarized some important signaling pathways, which provides direction for future research. In the KEGG pathway enrichment analysis, 17 (11.81%) gene targets were involved in MAPK signaling pathway, 17 (11.81%) gene targets were involved in PI3K-Akt signaling pathway, 14 (9.72%) gene targets were involved in Ras signaling pathway, and 6 (4.17%) gene targets were involved in VEGF signaling pathway, which suggest that common gene targets play a role in repairing fractures in multiple signaling pathways. MAPK and PI3K/AKT signaling pathways have been demonstrated to promote osteoblastic bone formation [40]. Zhang et al. confirmed that total flavonoids from DR promote the osteogenic differentiation of ciliary neurotrophic factor-modified myoblasts by activating p38 MAPK signaling pathway [41]. Moreover, total flavonoids of DR could promote osteogenic differentiation of rat dental pulp stem cells via the PI3K/Akt pathway [42]. Lin et al. found that the effect of naringin on the healing of fracture may be related to the promotion of the synthesis and secretion of cellular chemokines (CXCL5, CXCL6) and enhancement of mesenchymal stromal cell migration through Ras signaling pathway [43]. In addition, naringin stimulates angiogenesis by regulating the VEGF/VEGFR-2 signaling pathway in rats, thereby promoting fracture healing [38]. However, the mechanism of some active ingredients of DR in the treatment of fractures has not yet been verified. Therefore, we integrated MAPK, PI3K/AKT, Ras, and VEGF signaling pathways to provide a reference for researchers to verify the mechanism of other DR active ingredients in the treatment of fractures.

5. Conclusion

We collected the gene and protein targets of fractures and active ingredients of DR and then used network pharmacology to reveal the correlation between drugs and diseases and the potential synergistic mechanism of different targets of DR in the treatment of fractures, which provides a reference for the development of new drugs.

Abbreviations

DR:  Drynariae Rhizoma
GO:  Gene ontology
TCMSP:  Traditional Chinese Medicine Systems Pharmacology
OB:  Oral bioavailability
DL:  Druglikeness
CPI:  Chemical-protein interactome
PPI:  Protein-protein interaction
dock-:  Combining docking with intelligence
IN:  Integrated Discovery.

Data Availability

All data are available from the corresponding author upon reasonable request.
Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Haixiong Lin and Xiaotong Wang conceived and designed the study. Ziwei Jiang and Feng Huang revised the protocol. Haixiong Lin and Xiaotong Wang extracted the data. Ligang Wang, Hang Dong, Peizhen Huang, Qunbin Cai, and Yingjie Mo checked the data. Haixiong Lin, Xiaotong Wang, and Ligang Wang performed statistical analysis and wrote the manuscript. Haixiong Lin, Xiaotong Wang, Ligang Wang, Hang Dong, Peizhen Huang, Qunbin Cai, Yingjie Mo, Feng Huang, and Ziwei Jiang interpreted the results. Ziwei Jiang and Feng Huang reviewed and proposed advice. All authors contributed to constructive comments on the paper. Haixiong Lin, Xiaotong Wang, and Ligang Wang contributed equally to this work.

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Supplementary Materials

Supplementary Table 1: the topology properties of active ingredients of DR. (Supplementary Materials)

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