Predicting the levels of orcinol glucoside during the treatment of osteoporosis by network pharmacology and molecular docking

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Research

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Abstract

Introduction: As a traditional Chinese medicine (TCM), *Curculigo orchoides* Gaertn. (Xianmao) has been widely used to treat bone-related diseases. However, the active components of this TCM, and the specific mechanisms by which it exerts effect, have yet to be elucidated. To identify potential targets for orcinol glucoside (OG), an active constituent of *C. orchoides*, during the treatment of osteoporosis (OP) by adopting a network pharmacology approach.

Methods: First, we mined the Similarity ensemble approach (SEA), SwissTargetPrediction, DisGeNET, and Genecards databases were mined for data related to the prediction of OG- and OP-related targets. Next, we identified the common targets for OG and OP, and then used STRING software to create a protein-protein interaction (PPI) network. Then, we used topological analysis to identify which of the common targets were most significant. Then, we used the common significant targets and g:profiler to perform gene ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Finally, we used molecular docking to predict the targets of OG that were most relevant to the treatment of OP and investigated the potential pharmacological mechanisms that might be involved.

Results: In total, 130 potential targets of OG, and 4582 targets relevant to OP, were subjected to network analysis. There were 73 common targets; these identified the principal pathways linked to OP. In addition, topological analysis identified 14 key targets. Most of the predicted targets played crucial roles in the PI3K-AKT signaling pathway. Molecular docking identified ten core targets (*VEGFA*, *IL6*, *EGFR*, *MAPK1*, *HRAS*, *CCND1*, *FGF2*, *IL2*, *MCL1* and *CDK4*), thus indicating that OG may promote osteoblast proliferation and differentiation by accelerating progression of the cell cycle.

Conclusions: This research provides a theoretical base for identifying the specific potential mechanisms of OG in treatment of OP.

Introduction

Osteoporosis (OP) is a common metabolic bone disease that is characterized by low bone mineral density and microarchitectural changes in the bone, thus resulting in debilitating fragility and fractures [1]. OP is a global health issue that shows a close association with the aging population. Current estimates show that the population of patients with OP in China will increase by 60 million to over 120 million by the year 2050 [2]. The pathogenesis of OP is very complicated, including physical variables, nutritional status, endocrine factors, genetic factors, and a range of immune factors [3]. Numerous clinical therapies have been applied to treat OP, including calcium and vitamin D, bisphosphonates, anti-RANKL, selective estrogen receptor modulators, anabolic agents, and sclerostin inhibitors [4]. However, these drugs can only partially ease bone loss; their lasting clinical use is limited by high costs and low tolerability [5].

Traditional Chinese medicine (TCMs), such as Epimedium (Yinyanghuo), *Curculigo orchoides* Gaertn. (Xianmao), have been used for many years to treat OP [6]. The effects of water extract from epimedium

treatment on osteoporosis can be mitigated through a mechanism associated with several neuropeptides that regulate the brain/spinal cord/bone axis [7]. *C. orchioides* ethanol extract can inhibit bone absorption in rats undergoing oophorectomy, increase serum phosphorus and calcium levels, and have a certain protective effect on osteoporosis; however, this product does not affect bone formation [8].

Phytochemical studies previously demonstrated that *C. orchioides* contains an abundance of phenols and phenolic glycosides, triterpenes and triterpenoid glycosides, lignans, lignan glycosides, and many other types of compounds [9]. OG is one of the major bioactive phenolic glycosides of *C. orchioides* and is reported to have a wide range of pharmacological actions in mouse models, including antiosteoporosis [10], anxiolytic [11] and antidepressive [12] effects. The specific mechanisms underlying the therapeutic effects of OG in patients with OP, however, has yet to be elucidated. The role of OG in the treatment of OP can be better understood by detailed studies on molecular targets and related signal pathways.

With the rapid progression of bioinformatics technology, the development of network pharmacology has proven to be an innovative method for investigating the effects of TCM [13–14]; this new discipline involves network analysis, molecular docking, experimental methods, and integrates multiple information sources [15]. Therefore, network-based methods are expected to significantly enhance our understanding of drug effect by considering multiple sources of information [16].

In this research, we systematically explored the potential targets and molecular mechanisms of OG in the treatment of OP. First, we identified the molecular targets of OG and then predicted targets of disease using different types of bioinformatics platforms. Second, we selected the most significant common targets for OG and OP used STRING software to create a protein-protein interaction (PPI) network; Cytoscape was used to visualize the results of PPI. Third, we carried out enrichment analysis of common targets according to gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Finally, the therapeutic effect of OG, and several key targets identified by topological analysis, was confirmed by molecular docking experiments.

Methods

The prediction of targets for OG

PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) is the world's largest free chemical database and offers information related to compound structures and biological activities. First, we used OG to search the PubChem database to acquire canonical SMILES strings. These strings were then sent to SwissTargetPrediction (<http://new.swisstargetprediction.ch/?>) and Similarity ensemble approach (SEA) (<http://sea.bkslab.org/>) to identify targets. In this part of the analysis, the species was set to “human” and the prediction results were collated and classified

Screening of targets related to OP

Disease-related gene screening was carried out by screening two free public databases: the DisGeNET database v6.0 (<https://www.disgenet.org/>) [17] and Genecards database (<https://www.genecards.org/>) [18] with the keyword “osteoporosis”. We also used UniProtKB (<https://www.uniprot.org/>) [19] to acquire the names of standard targets with “homo sapiens” as the selected organism.

After collating the targets and removing any duplicates, the final list of predicted targets were estimated to represent common targets. Next, the common targets of OG that showed relevance with regards to its effects on OP were identified. We were particularly interested in targets that may play an active role in the proliferation and differentiation of osteoblasts by accelerating cell cycle progression.

Construction of a visualization network

The identified targets were then inputted into the STRING database v11.0 (<https://string-db.org/>) [20] for PPI analysis, including their physical and functional associations. During this analysis, we set the score > 0.4 medium confidence, and the results were exported in tab-separated value (TSV) format. PPI results were subsequently imported into Cytoscape 3.7.2 [21] to visualize the results.

Next, we used Network Analyzer to calculate the network topological parameters by treating the network as undirected [22]. We also used the CytoNCA plugin [23], and the “without weight” method to determine the three centralities: degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC).

In this study, the nodes with a DC and BC score that were greater than the median by two-fold were taken as key targets in the network. We assumed that these were the key targets for anti-osteoporosis. In this experiment, the median DC was 8, and the median BC was 15.14135.

GO and KEGG pathway enrichment analysis

GO and KEGG pathway enrichment analyses was carried out for OG targets in the treatment of OP. To facilitate this analysis, we used a web server for functional enrichment analysis known as g:Profiler (<https://biit.cs.ut.ee/gprofiler/gost>) [24]. This analysis was performed and visualized by OmicShare tools, a free online platform for data analysis (<https://www.omicshare.com/tools/>).

Molecular docking

Three-dimensional (3D) structures of the ligand was obtained from PubChem. The crystal structures of key targets were then downloaded from the RCSB Protein Data Bank (<https://www.wwpdb.org/>) and modified using PyMOL [25], including solvents, ligands, and original ligands. The key target proteins were as follows: VEGFA (PDB ID: 3QTK), IL6 (PDB ID: 4O9H), EGFR (PDB ID: 3IKA), MAPK1 (PDB ID: 4FV4), HRAS (PDB ID: 6ZL3), CCND1 (PDB ID: 6P8E), FGF2 (PDB ID: 5X1O), IL2 (PDB ID: 4NEM), MCL1 (PDB ID: 4WMR), and CDK4 (PDB ID: 2W99). Next, Autodock Vina (Olson Research Group, Scripps Research Institute) was used to analyze the binding characteristics of the ligand for each protein [26]. First, we used Autodock Tools to save the proteins and ligand in PDBQT formats [27]; the grid box parameters are shown in Table 1. Exhaustiveness was set to the value of 60 for all docking analysis. Other settings were

all set as default values. After docking, we selected the lowest binding affinity score for each protein for further analysis. Ligplus [28] and PyMOL were used to visualize the interactions between OG and the key targets as two-dimensional (2D) and three-dimensional (3D) graphs.

Table 1 Grid box parameters used for molecular docking

<i>Targets</i>	<i>PDB ID</i>	<i>Grid Center</i>			<i>Npts</i>	<i>Spacing</i>
VEGFA	3QTK	24.624	-1.635	-7.692	60 60 60	1.000
IL6	4O9H	-34.017	23.971	11.472	60 60 60	1.000
EGFR	3IKA	-10.730	27.973	36.465	60 60 60	1.000
MAPK1	4FV4	-16.691	3.162	15.987	60 60 60	1.000
HRAS	6ZL3	29.700	-22.911	14.229	60 60 60	1.000
CCND1	6P8E	31.466	12.636	44.714	60 60 60	1.000
FGF2	5X10	31.752	2.507	261.200	60 60 60	1.000
IL2	4NEM	-14.928	8.061	18.532	60 60 60	1.000
MCL1	4WMR	-3.495	-12.165	7.518	60 60 60	1.000
CDK4	2W99	29.545	19.537	14.264	60 60 60	1.000

Results

Screening of potential targets

After the elimination of duplicates, SEA and SwissTargetPrediction databases identified a total of 130 predicted targets for OG (Table S1). DisGeNET and Genecards databases further identified a total of 4582 targets associated with OP (Table S2). The intersection between these two lists of targets identified 73 common targets of OG that were associated with OP.

OG-OP target network

Based on the 73 common targets, we created a PPI network by importing the genes of the common targets into the STRING database. Then, Cytoscape 3.7.2 software was used to visualize the PPI network, which consisted of 73 nodes and 461 edges (Fig. 1). According to strict criteria (twice the median of DC and BC), 14 critical nodes (“key targets”) were further identified, as shown in Table 2. These 14 key targets may represent valuable targets for OG in the treatment of OP.

Table 2
Topological parameters of the targets

<i>Gene</i>	<i>DC</i>	<i>BC</i>	<i>CC</i>
GAPDH	49	1058.475	0.75
VEGFA	45	640.9635	0.72
IL6	44	763.3865	0.72
EGFR	41	440.131	0.692308
MAPK1	33	279.9036	0.631579
HRAS	32	154.2462	0.631579
MMP9	30	305.9342	0.615385
CCND1	30	90.02024	0.615385
ESR1	29	272.7521	0.605042
FGF2	27	124.6053	0.6
IL2	25	118.7386	0.595041
MCL1	22	51.10877	0.571429
CDK4	20	35.86626	0.5625
F2	16	93.15169	0.541353

GO terms and KEGG pathway enrichment analyses

Next, the 73 common targets were imported to the g: Profiler for GO and KEGG analysis. GO terms and KEGG pathways with a p -value < 0.05 were considered to be notably enriched. The top 20 elements were then visualized using OmicShare tools (Fig. 2).

As shown in Fig. 2, the top five items of biological processes (each with a p value < 0.05) included organonitrogen compound metabolic process, cell population proliferation, protein metabolic process, regulation of response to stress, and response to chemicals. The top five items for the cellular components category included cyclin-dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, protein kinase complex, extracellular region, and extracellular space. The top five items for the molecular functions category included catalytic activity, catalytic activity, acting on a protein, protein kinase activity, metalloendopeptidase activity, and serine-type endopeptidase activity.

KEGG analysis further showed that 54 of the 73 common targets (74.0%) were notably enriched in 51 pathways (Table S3). Among the enriched pathways, we identified abnormalities in terms of the PI3K-AKT signaling pathway in OP. The predicted targets that refer to the PI3K-AKT signaling pathway are indicated in red in Fig. 3.

Molecular Docking

Ten select key targets, *VEGFA*, *IL6*, *EGFR*, *MAPK1*, *HRAS*, *CCND1*, *FGF2*, *IL2*, *MCL1* and *CDK4*, were obtained because they were not only the key targets of PPI network, but also played a major role in the PI3K-AKT signaling pathway. These genes were subjected to OG for molecular docking. As summarized in Table 3, the binding energies were computed to evaluate the binding affinities of the ten targets with OG. A binding affinity < -7 showed strong binding activity [26].

Table 3
Molecular docking results between orcinol glucoside and key targets

<i>Targets</i>	<i>PDB ID</i>	<i>Binding Energy (ΔG)/kcal·mol⁻¹</i>
VEGFA	3QTK	-7.3
IL6	4O9H	-6.7
EGFR	3IKA	-7.0
MAPK1	4FV4	-7.2
HRAS	6ZL3	-7.4
CCND1	6P8E	-7.0
FGF2	5 × 10	-7.9
IL2	4NEM	-6.1
MCL1	4WMR	-7.2
CDK4	2W99	-7.8

The mode of action for OG with VEGFA is shown in Fig. 4A; the hydroxyl group of OG is able to form two hydrogen bonds with the amino acid Asn55 (2.82 Å) and Asp56 (3.00 Å) within the active pocket. In addition, Cys53, Cys54, Phe29, Arg98, Lys101, Ile39, and Glu57, can all form hydrophobic interactions; these help to stabilize the entire region that is used for interaction. In addition, the results in Fig. 4B show that OG binds to IL6 by forming several hydrophobic interactions with the surrounding residues: Lys46, Arg104, Leu39, Leu101, and Ala112.

As shown in Fig. 4C, we observed hydrophobic interactions between OG and seven residues in 3IKA (Leu718, Ala743, Leu844, Leu792, Gly796, Val726, and Met790). Two hydrogen bonds, Met793 (3.10 Å) and Lys745 (2.92 Å), further enhanced the interaction between OG and the 3IKA protein.

Figure 4D shows that OG binds to MAPK1 by forming several hydrophobic interaction with several surrounding residues: Cys164, Ala50, Leu154, Val37, Ile29, and Leu105. Analysis also showed that OG forms three H-bonds with Met106 (3.05 Å), Gln103 (3.17 Å) and Lys52 (3.34 Å).

The interplay between OG and HRAS is shown in Fig. 4E; three H-bonds were observed with Lys117 (2.80 Å), Ala146 (3.02 Å), and Lys147 (3.02 Å), in the binding pocket of HRAS. In addition, hydrophobic

interactions with five residues in HRAS (Phe28, Gly15, Ala18, Asn116, and Asp30), along with pi-pi stacking with Phe28, further facilitated the binding of OG to HRAS.

As shown in Fig. 4F, OG interacted with CCND1 by Asn221, Pro220, Arg218, Leu217, Ile177, and Gly214. Furthermore, OG can form H-bonds with Asn174 (3.22 Å), His181 (3.00 Å), and Asn222 (3.16 Å).

Figure 4G shows that OG binds to a pocket in FGF2 which consists of Pro36, His50, Ile51, Val40, Lys66, Tyr73, and Ala84. Furthermore, OG can form two H-Bonds with Leu74 (2.94 Å), and Gly67 (3.14 Å).

The data shown in Fig. 4H further shows that OG can form hydrophobic interactions with four residues in IL2 (Pro34, Ile28, Ile24, and Gln74) and one H-Bond (Asn30 (3.33 Å)).

Figure 4I show the binding ability between OG and MCL1 (Fig. 4I) which involves hydrophobic interactions with Phe254, Val253, Thr266, Leu267, Gly271, Ile294, Leu290, Met250, and Val249. In addition, one H-Bond with Arg263 (3.30 Å), along with pi-pi stacking with Phe270, further facilitated the binding of OG to MCL1.

The docking results between OG and CDK4 are shown in Fig. 4J. OG binds to a pocket in CDK4 which consists of Ile12, Glu144, Val20, Asn145, Phe93, Ala157, Leu147, Val72, and Ala33. Four H-Bonds (Asp158 (3.11 Å), Lys35 (3.02 Å), Val96 (3.17 Å), and His95 (3.28 Å)) further enhance the interaction between OG and the CDK4 protein.

Discussion

With the increasing incidence of OP and the poor therapeutic efficacy of clinical drugs, there is an urgent need to develop new treatment strategies. Therefore, the search of complementary and alternative medicine has become our top priority. As a famous kidney-tonifying traditional medicine, *C. orchioides* has been widely used against OP. The major active constituent of OG is *C. orchioides*; previous research has shown that *C. orchioides* exerts significant effects against OP [10]. Here, we used network pharmacology and molecular docking approaches to investigate existing literature and confirm potential mechanisms underlying the role of OG.

We hypothesized that OG can be used as a therapeutic for the treatment of OP and that OG promotes the proliferation and differentiation of osteoblasts by accelerating cell cycle progression. We then carried out a series of investigations to test this hypothesis. Another active molecule, Curculigoside, also isolated from *C. orchioides* has been reported to have a similar therapeutic mechanism and exhibits anti-OP effects by inducing the proliferation and differentiation of osteoblasts [29] and by reducing the inflammatory response [30].

Enrichment analysis of KEGG pathways showed that OG targets were enriched in the PIK3-AKT signaling pathway. Previous research also showed that the activation of the PI3K-AKT signaling pathway exhibits a strong correlation with the occurrence and development of OP [31]. During the pathogenesis of OP, the PIK3-AKT pathway contributes to the progression of disease through cell survival, proliferation,

differentiation, and apoptosis [32]. Therefore, targeting the PI3K/Akt signaling pathway may be a potential treatment for osteoporosis.

In this study, we first identified 73 common targets for drugs and diseases that might also represent targets for OG during the treatment of OP. Based on topological analysis, we further identified 14 key targets, including *GAPDH*, *VEGFA*, *IL6*, *EGFR*, *MAPK1*, *HRAS*, *MMP9*, *CCND1*, *ESR1*, *FGF2*, *IL2*, *MCL1*, *CDK4*, and *F2*. Among the top 20 enriched KEGG pathways, the PI3K-AKT signaling pathway was shown to be particularly important as abnormalities were clearly evident in OP. It was evident that the key target genes were not exactly the same as those in the PI3K-AKT signaling pathway. Proteins that were confirmed as key targets and involving the PI3K-AKT signaling pathway were thus selected for molecular docking.

In total, ten targets were selected for the molecular docking studies: *VEGFA*, *IL6*, *EGFR*, *MAPK1*, *HRAS*, *CCND1*, *FGF2*, *IL2*, *MCL1* and *CDK4*. Cell proliferation and differentiation can be adjusted via alterations of the cell cycle phase, thus causing indirect effects on bone formation [33]. Our molecular docking studies suggest that OG can affect the osteoblast cell cycle and has strong affinity for *CCND1* ($-7.0 \text{ kcal}\cdot\text{mol}^{-1}$), and *CDK4* ($-7.8 \text{ kcal}\cdot\text{mol}^{-1}$); these are key players in cell cycle progression. Consistent with this hypothesis, pharmacological studies have consistently shown that related compounds can downgrade *CCND1* or *CDK4* to repress osteogenic proliferation and differentiation [34–35]. Studies have also shown that *VEGFA*, *EGFR*, *MAPK1*, and *FGF2* play a critical role in the proliferation and differentiation of osteoblast cells [36–39]. In this study, we found that OG may activate *VEGFA*, *EGFR*, and *MAPK1*, as their binding affinities for interaction were all <-7.0 . Furthermore, *IL6* and *IL2* are involved not only in inflammatory responses but also in the regulation of bone mineral density, osteoclast differentiation and activation [40–41]. However, our molecular docking results for *IL6* and *IL2* showed binding affinities of -6.7 and $-6.1 \text{ kcal}\cdot\text{mol}^{-1}$; these are higher than $-7.0 \text{ kcal}\cdot\text{mol}^{-1}$ and therefore indicate that OG exerts only weak inhibition on inflammatory responses.

Conclusion

By combining network pharmacology and molecular docking, we showed that OG is a promising treatment for OP and acts via several key targets (*VEGFA*, *IL6*, *EGFR*, *MAPK1*, *HRAS*, *CCND1*, *FGF2*, *IL2*, *MCL1* and *CDK4*). Via these targets, OG may promote the proliferation of osteoblast by altering cell cycle progression. However, further experimental testing is now required to investigate the specific mechanisms underlying the action of OG on OP.

Abbreviations

OG: orcinol glucoside; OP: osteoporosis; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BC: betweenness centrality; CC: closeness centrality; DC: degree centrality; Npts: number of points in x-, y-, z-dimension; *VEGFA*: Vascular endothelial growth factor A; *IL6*: Interleukin-6; *EGFR*: Epidermal growth factor receptor; *MAPK1*: Mitogen-activated protein kinase 1; *HRAS*: GTPase HRas;

CCND1: G1/S-specific cyclin-D1; FGF2: Fibroblast growth factor 2; IL2: Interleukin-2; MCL1: Induced myeloid leukemia cell differentiation protein Mcl-1; CDK4: Cyclin-dependent kinase 4.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials used or analyzed during this current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MZ designed the study. XL, MH, CY and QW carried out the experimental work., MZ, XL and MH analyzed the experimental data and wrote the paper. All authors reviewed the manuscript and approved its submission.

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Figures

Figure 1

The orcinol glucoside-osteoporosis target network. The color and size of each circle reflects the node degree for the common targets.

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GO terms and KEGG pathway enrichment analysis of 73 common targets ($p\text{-value} < 0.05$). (A) The top 20 biological processes. (B) The top 20 cellular components. (C) The top 20 molecular functions. (D) The top 20 KEGG pathways. The color scales indicate different thresholds for p-values while the sizes of the dots represent the number of genes corresponding to each term

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