Study on the action mechanism of Wuling Powder on treating osteoporosis based on network pharmacology

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[ABSTRACT] Osteoporosis is a health problem to cause global concern. A lot of methods have been used to prevent and treat osteoporosis, but there is still a lack of effective treatment for osteoporosis owing to limited understanding of its mechanism. Therefore, the aim of this present study is to explore the underlying mechanism of Wuling Powder, a traditional Chinese medicine on treating osteoporosis. In this study, we firstly screened and identified the common targets between Wuling Powder and osteoporosis through the related databases, and then explored the relationships among these targets, Wuling Powder and osteoporosis by using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and network analyses. Subsequently, the molecular docking was performed by using systemsDock to evaluate the potential binding relationships between the active components of Wuling Powder and their related targets. The results showed that in total of 14 common targets including CREBBP, ADAM17, GOT1, GAPDH, USP8, ERBB2, EEF1A1, MTOR, RAC1, ETS1, DDX58, GCK, EGF and S100A8 were screened. EGF, ERBB2, MTOR and HIF-1 were the potential therapeutic targets for osteoporosis, and they were also the related targets for predicting active components in Wuling Powder. Taken together, we concluded that Wuling Powder might be used to treat osteoporosis through above these targets.

[KEY WORDS] Wuling Powder; Osteoporosis; Network Pharmacology

Introduction

Osteoporosis is a health problem to cause global concern, and the osteoporotic fracture is a major factor causing disability and medical costs around the world [1]. Early diagnosis and prophylactic drug are the key to prevent and treat osteoporosis and reduce osteoporotic fracture for the high-risk patients [1]. At present, the treatment of osteoporosis has achieved a certain degree of consensus, but there was still some controversy, and novel treatment methods were constantly emerging [1-9]. In general, traditional Chinese medicine (TCM) and plants are the abundant resource for active components of some drugs. Therefore, we hope to find a novel prevention and treatment method for osteoporosis from TCM, thereby enriching the existing treatment methods.

Wuling Powder, a TCM, was also called Wuling San. It primarily derives from mycelia of Xylaria nigripes Sacc., and has been proved to can treat insomnia and depression and improve cognitive deficit [6]. According to the western medicine, estrogen deficiency was often thought to be a major pathogenetic factor to cause osteoporosis [7]. However, Chinese medicine theory thought that the kidney could store essence and transform the essence into bone marrow thereby nourishing the bones, and the TCM for tonifying kidney could markedly increase estrogen level thereby alleviating osteoporosis [8]. Therefore, we thought that the experimental method for western medicine would contribute to explore the action mechanism of a TCM, and even bring more in-depth insights into the action mechanism of Wuling Powder on the treatment of osteoporosis.

In this present study, the network pharmacology was used to investigate the action mechanism of Wuling Powder
on treating osteoporosis. The recent studies showed that *Wuling* Powder could play roles in the depression-like behavior via enhancing the translocator protein-mediated mitophagy [8], prevent kidney dysfunction through suppressing renal toll-like receptor 4/myeloid differential protein-88 pathway and the activation of pyrin domain containing 3 inflammasome [9], and could also play an anti-depressant role via L-arginine-nitric oxide-cyclic guanosine monophosphate pathway [10]. However, few literatures about the action mechanism or pathway of *Wuling* Powder on treating osteoporosis were reported. Therefore, we firstly screened and identified the common targets between *Wuling* Powder and osteoporosis through the related databases, and then explored the relationships among these targets, *Wuling* Powder and osteoporosis by using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and network analyses, thereby obtaining the key therapeutic targets for osteoporosis.

**Materials and Methods**

**The exaction of active components from *Wuling* Powder**

In this study, the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) database (http://lsp.nwu.edu.cn/tcmsp.php) was used to investigate the pharmacokinetic characteristics of active components of *Wuling* Powder, in order to obtain their information about the absorption, distribution, metabolism and excretion (ADME) characteristics of drugs with potential biological effects, such as oral bioavailability (OB), drug likeness (DL), Caco-2 permeability (Caco-2), blood-brain barrier (BBB), and so on [11]. Subsequently, the active components were collected according to the previously proposed screening criteria that included oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 [12-13].

**The obtaining of related targets for active components**

The potential targets were derived from the PharmMapper database (http://lilab.ecust.edu.cn/pharmmapper/), which was designed to identify the potential targets for small molecules by using a reverse pharmacophore mapping approach [14]. The top 15 potential targets for each component were obtained and screened by using the fit score value. Meanwhile, the repetitive and unhuman targets were excluded. **The obtaining of related targets for osteoporosis**


**The screening and identification of common targets**

Common targets for active components and osteoporosis were obtained by using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

**GO and KEGG analyses**

GO analysis consists of biological process (BP), cellular component (CC) and molecular function (MF) terms, and KEGG signaling pathway analysis was derived from the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) [18], and data were analyzed with GraphPad Prism software version 8. A bubble chart was plotted by using the OmicShare (http://www.omicshare.com/tools). A *P* value ≤ 0.05 was considered significant, and the enriched GO terms were identified by using a hypergeometric test.

**The establishment of protein-protein interaction (PPI) network**

The PPI Network was established by using Cytoscape v3.6.1 in order to explain the relationships among the proteins.

**Molecular docking**

The molecular docking was performed by using the crucial proteins with high degree based on the PPI network. The lowest energy conformations were adopted for molecular docking via default parameters in systemsDock (http://systemsdock.unit.oist.jp/iddp/home/index) [19-20]. Docking score represented a negative logarithm of experimental dissociation/inhibition constant value (pKd/pKi) usually ranging from 0 to 10 (from weak to strong combining ability).

**Results**

**Common targets for *Wuling* Powder and osteoporosis**

In total of 47 active components of *Wuling* Powder were obtained from TCMSP. Then, the component-related targets were predicted by using PharmMapper, and 85 targets were obtained after removing the repetitive and unhuman ones. Using CTD and GeneCards, 795 targets were gathered after removing duplicates. Hence, in total of 14 common targets between *Wuling* Powder and osteoporosis were screened and identified, including CREBBP, ADAM17, GOT1, GPDH, USP8, ERBB2, EEF1A1, MTOR, RA C1, ETS1, DDX58, GCK, EGF and S100A8 (Fig. 1).

**GO and KEGG analyses**

The DAVID database was used to perform GO analysis on above these mentioned 14 potential targets. The results showed that GO terms were mainly divided into three parts: BP, CC and MF (Fig. 2, Table 1). The BP was associated with the activation of positive regulation of cell proliferation, positive regulation of mitogen-activated protein kinase activity, epidermal growth factor (EGF) receptor signaling pathway, positive regulation of translation, positive regulation of
actin filament polymerization, gluconeogenesis, positive regulation of stress fiber assembly, cell proliferation, glycolytic process, cellular response to EGF stimulus, regulation of angiogenesis, regulation of cell motility, canonical glycolysis, positive regulation of dendritic spine development, positive regulation of gene expression, positive regulation of lamellipodium assembly, ruffle organization, positive regulation of transcription from RNA polymerase III promoter, positive regulation of cellular component movement, response to hypoxia, positive regulation of EGF-activated receptor activity, Notch signaling pathway, phosphatidylinositol-mediated signaling, positive regulation of cell growth, wound healing, ErbB2 signaling pathway, cell motility and positive regulation of protein phosphorylation. The CC was associated with the extracellular exosome, extrinsic component of plasma membrane, nucleus, membrane, cytosol, cytoplasm and ruffle membrane. Meanwhile, MF was related to phosphatidylinositol-4,5-bisphosphate 3-kinase activity, protein kinase binding, protein binding and identical protein binding.

Through comprehensive analysis, first 12 Wuling Powder-related KEGG signal pathways were obtained, and the KEGG signal pathway for Wuling Powder was constructed according to the $P$ value on a bubble plot (Table 2). Subsequently, based on the systems-level image, an important signal pathway, hypoxia-inducible factor-1 (HIF-1) signal pathway, was selected for the further analysis (Fig. 3).

**Network analysis**

After obtaining the active components-related targets in Wuling Powder, a PPI network for these targets was established as shown in Fig. 4, which reveals the significant relationships among EGF, MTOR, EIF4E and RPS3. In order to show these complicated relationships, the PPI network between the active components and their related targets was also constructed (Fig. 5). Finally, 6 ones out of 14 common targets between Wuling Powder and osteoporosis were screened by regulating minimum required interaction score to the highest confidence (0.900), and then the PPI network was constructed (Fig. 6). It showed the significant connections among EGF, ERBB2, ADAM17, RAC1, MTOR and USP8.

**Molecular docking**

Molecular docking analysis demonstrated the potential binding relationships between these active components and their related target genes. A docking score of $> 4.25$ was considered fair, $5 \leq$ docking score $< 7$ was considered good, and docking score $\geq 7$ was considered excellent; this scoring was conventionally used to classify ligand binding activity. 

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**Fig. 2** The terms for biological process, cell component and molecular function with $P < 0.05$ were shown.
Among them, Wuling Powder was successfully combined with three key genes including EGF, MTOR and RAC1, and the docking score was between 4.777 and 7.982, indicating that EGF, MTOR and RAC1 may have the strong binding ability to Wuling Powder (Fig. 7).

**Discussion**

There is a delicate balance between osteoblast and osteoclast in the healthy bones, which is regulated by a variety of molecules and genes [21-22]. So some drugs for these molecules or genes may be used to prevent and treat osteoporosis. In our study, Wuling Powder was proved to directly or indirectly influence the expressions of some important proteins, especially four factors including EGF, ERBB2, MTOR and HIF-1. This finding is reported for the first time, and these four targets may be related to the action mechanism of Wuling Powder on the treatment of osteoporosis.
EGF, a single-chain polypeptide derived from the cleavage of a large precursor, is consisted of pre-epidermal growth factor composed of 53 amino acids [23]. A previous study indicated that some plant extracts could modulate the EGF signaling pathway, and were associated with the reduced incidence of osteoporosis [24]. In addition, the EGF signaling pathway was also proved to stimulate the migration of osteoblasts [25], and abnormal apoptosis of osteoblasts would contribute to the genesis of osteoporosis [7]. Human ERBB2 belongs to EGF receptor (EGFR) family, and there are about four members in the EGFR class named as EGFR/ERBB1, ERBB2/Neu/HER2, ERBB3 and ERBB4 [26]. A previous report indicated that EGF and ERBB2 were all relevant in healthy bone remodeling and fracture healing and could also be impaired in pathology like in osteoporosis [27]. Therefore, Wuling Powder may reduce the incidence of osteoporosis through regulating the EGF- and ERBB2-related signaling pathways.

Fig. 3 The “Rich factor” represented the ratio of the number of target genes belonging to this pathway and the number of the annotated genes located in a pathway. A high rich factor represented a high level of enrichment. The size of the dot meant the number of target genes in the pathway, and the color of the dot reflected the P value.

Fig. 4 The construction of PPI network of proteins expressed by Wuling Powder. The 36 nodes represented 36 proteins, and 39 lines represented 39 pairs of interaction among these proteins. The node size and color represented the degree, while line size and color represented the combined score. All data were from STRING.
As we all known, the imbalance between bone formation and resorption is the main reason to cause osteoporosis. A recent research has revealed that autophagy was involved in the maintenance of bone homoeostasis, and proposed that autophagy was associated with osteoporosis. MTOR, a well-known pharmacologic target for autophagy, played a crucial role in autophagy regulation. Thus, given that Wuling Powder was proved to influence the expression of MTOR in this study, we propose that Wuling Powder may also influence the incidence of osteoporosis via the MTOR target.

A previous study indicated that the MTOR pathway could also regulate the activation of HIF-1 in osteoblasts, and EGFR might down-regulate the expression of HIF-1.
Therefore, these four targets including EGF, ERRB2, MTOR and HIF-1 could interact and together play the related roles in the treatment of osteoporosis.

In conclusion, EGF, ERRB2, MTOR and HIF-1 were all the potential therapeutic targets for osteoporosis, and they were also the related targets for predicting active components in Wuling Powder. Therefore, we concluded that Wuling Powder might be used to treat osteoporosis through above these targets.

Estrogen is effective in the treatment of osteoporosis, especially in women with postmenopausal osteoporosis, but direct use can induce breast cancer and increase the risk of cerebrovascular accidents and venous thromboembolism [9]. Through this study, we hope to demonstrate the theoretical basis of Wuling Powder in the treatment of osteoporosis, so as to provide a direction for further experiments, speed up the progress of clinical trials, and provide a new and less likely side effect treatment for patients with osteoporosis as soon as possible.

Conclusions

Wuling Powder may treat osteoporosis through EGF, ERRB2, MTOR and HIF-1 pathways.

Abbreviations:

ADAM17, ADAM metallopeptidase domain 17; CREBBP: CREB binding protein; DDX58: DExD/H-box helicase S8; EEF1A1: eukaryotic translation elongation factor 1 α 1; EGF, epidermal growth factor; ERRB2: erb-b2 receptor tyrosine kinase 2; ETS1: ETS proto-oncogene 1, transcription factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GCK: glucokinase; GOT1: glutamic-oxaloacetic transaminase 1; MTOR: mechanistic target of rapamycin kinase; RAC1: Rac family small GTPase 1; S100A8: S100 calcium binding protein A8; USP8: ubiquitin specific peptidase 8.

References


