Systematic elucidation of the molecular mechanism of scutellarin against osteoarthritis based on network pharmacology

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Research

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Abstract

Background

Scutellarin was reported to exerted inhibitive effects on osteoarthritis, However, the detailed mechanisms remain unclear. In this study, we investigated underlying multi-target mechanisms of scutellarin against osteoarthritis by using network pharmacology analysis and molecular docking.

Results

Scutellarin exerted inhibitive effects on osteoarthritis by regulating the function of several new signaling pathways, such as TNF signaling pathway, NOD-like receptor signaling pathway and HIF-1 signaling pathway. Molecular docking analysis showed there was better interaction between scutellarin and several NF- κ B signaling proteins, including NFKBIA, RELA and NFKB1. In addition, the results showed Pi-cation, Pi-donor-hydrogen and Pi-alkyl were the main forms of interaction between scutellarin and NFKB1 and NFKBIA, Pi-Pi T-shaped, Pi-alkyl and hydrogen bonding were the main forms of interaction between scutellarin and RELA.

Conclusion

Taken together, TNF signaling pathway, NOD-like receptor signaling pathway and HIF-1 signaling pathway were possible signaling pathways, NFKBIA, RELA and NFKB1 were possible targets associated with the activities of scutellarin against osteoarthritis. However, it is imperative that these targets should be thoroughly verified by in vitro and in vivo experiments.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease among the aged, and a major cause of severe joint pain and disability[1], which is characteristic of articular cartilage destruction, subchondral bone rebuilding and inflammation of the synovium [2, 3]. Although the understanding of OA has been improved in recent years, there is still lack of effective drugs to delay and reverse the pathological process of OA, so that patients in advanced stages have to accept arthroplasty, which cause heavy burdens on society. Hence, it is urgent to find a new effective and low-cost medicine for OA[4, 5]. Previous studies have shown that the development of OA is closely related to aging, obesity, abnormal metabolism, osteoporosis, and joint malformation [6, 7]. Therefore, an effective treatment of OA must consider all these factors and the molecular networks behind them.

Scutellarin (SCU) (4',5,6-trihydroxy flavonoid-7-glucuronide), a flavonoid glycoside isolated from *Erigeron breviscapus*, exhibits anti-inflammatory, anti-oxidative, and anti-apoptotic activity in various pathological processes, including cerebrovascular disease, dermatitis, atherosclerosis, and myocardial infarction[8–

10]. Recently, several studies have showed Scutellarin exerted inhibitive effects on the OA[11–13]. The underlying anti-OA mechanisms played by Scutellarin are closely related with several biological signal pathways, including Nrf2 pathway, Wnt/ β -catenin pathway, MAPK pathway, NF- κ B pathway and PI3K/AKT pathway[11–13]. However, the detailed mechanisms of scutellarin against OA remain unclear.

With the development of bioinformatics, the study of disease processions and molecular mechanisms by constructing and detecting biological network is considered as a novel method that can effectively elucidate mechanism of action, which provides a new option for strategy transformation from one-target to multiple-target and for the discovery of new therapeutic targets[14, 15].

In present study, we investigated potential mechanisms of Scutellarin against OA by using network pharmacology and molecular docking. Our results may be helpful for systematic clarification of molecular mechanisms of Scutellarin against OA and facilitate the development of Scutellarin as a novel drug for OA.

2. Materials And Methods

2.1 Predicting potential targets of scutellarin against OA

Targets corresponding with scutellarin were collected from the following databases. GenCLiP3[16] (<http://ci.smu.edu.cn/genclip3/analysis.php>), PharmMapper[17] (<http://www.lilab-ecust.cn/pharmmapper/>), Swiss Target Prediction[18] (<http://www.swisstargetprediction.ch/>). The 'scutellarin' was submitted to search the related targets in GenCLiP3 database. The SDF format of Scutellarin (PubChem CID: 185617) was imputed to PharmMapper to obtain targets using the pharmacophore mapping approach. And in Swiss Target Prediction, we use SMILES string of scutellarin(C1=CC(=CC=C1C2=CC(=O)C3=C(C(=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O)O) to get the targets. There were 439 targets associated with scutellarin after removing repeat targets, and the specific information about genes was shown in supplementary Table 1. Genes related to OA were derived from the GenCLiP3 databases. 'Osteoarthritis' was used as the search term. There were 1594 targets in final list of genes linked with OA after deleting duplicates, and the information regarding these targets is provided in supplementary Table 2.

2.2 Construction of PPI network and topological analysis

Targets associated with scutellarin and OA collected from the various major databases were intersected to get the predicted common genes. The interaction network of common genes was constructed by using String database[19] and visualized in the Cytoscape3.7.2[20]. Network analyzer in Cytoscape was used to analyze topological parameters in PPI network. Then, the core targets were screened according to degree, with the upper limit of the screening range was the maximum degree value, and the lower limit was twice the median degree value.

2.3 Cluster analysis

Molecular Complex Detection Algorithm (MCODE), a software in Cytoscape3.7.2, was used to identify the important cluster in this PPI interaction network of 138 genes, which can also detect densely connected regions in PPI networks[21]. The criteria settings are degree cutoff = 2, node score cutoff = 0.2, K-core = 2 and Max depth = 100 to acquire the most valuable cluster. Afterward, the core modules networks were obtained.

2.4 Genotype-phenotype correlation analysis

VarElect[22] was used to explore the correlation between the predicted core targets and OA, which can quickly identify genes directly or indirectly related to a certain phenotype/disease and rank the genes.

2.5 Biological functions analysis

DAVID[23] was used to perform pathway enrichment analysis and R software (R3.6.3 for Windows, ggplot2 package) was used to visualize the core terms. Interaction networks between signal pathways was constructed by Cytoscape.

2.6 Molecular docking

In order to obtain the internal specific relationship, molecular docking was performed to assess the strength and mode of interactions between Scutellarin and the predicted targets. RCSB Protein Data Bank(PDB:2AZ5, 1SVC, 6Y1G,3QXY) were used to search the crystal structures of hub protein which may be the most potential targets of Scutellarin against Osteoarthritis, and then we remove waters, add hydrogen and patching to modify the crystal structure by Autodock[24, 25]. The Scutellarin structure was downloaded from PubChem, and the three-dimensional chemical structure was obtained by ChemBioDraw3D. Then, the molecular docking of Scutellarin and the hub targets were done by Autodock Vina[26] and Discovery Studio software, and visualized by PyMOL. Meanwhile, the affinity and the mode of action were analyzed.

3. Results

3.1 Collection of candidate targets of scutellarin against OA

The workflow diagram of this study was shown in Fig. 1. As results, 1594 OA associated genes were collected by using GenCLiP3 database. In addition, a total of 164 predicted targets were screened from GenCLiP3 database, 207 predicted targets were harvested from PharmMapper database and 101 predicted targets were gained from Swiss Target Prediction database. Finally, 171 candidate targets of scutellarin against OA were collected (Fig. 2).

3.2 Construction of PPI network and topological analysis

Further, 171 candidate proteins were used for the following construction of PPI networks. Under the confidence score above 0.9, 138 nodes and 761 edges were identified by String database, and the protein network was visualized by using Cytoscape (Fig. 3).

3.3. Topological network analysis and identification of core targets

Network analyzer in Cytoscape software was used to assay the topological parameters of the PPI. The calculated median degree was 8, and the maximum degree was 47. Therefore, the degree in the screening conditions of core targets were ranged from 16 to 47. As results, the 34 core target proteins were obtained and PPI network was visualized (Fig. 4).

3.4 Correlation analysis between candidate targets and Osteoarthritis

The 34 core genes were input into the VarElect to analyze genotype-phenotype correlations. As shown in Table 1, the 33 genes have the direct correlation with Osteoarthritis, IL1B, TNF, IL6, MMP9 and VEGFA have best correlation in these gene, with the score is 26.8, 25.19, 24.62, 18.65 and 10.75, respectively.

Table 1
VarElect analysis of targets of scutellarin with the OA phenotype

NO.	Symbol	Description	Direct/Indirect	Score
1	IL1B	Interleukin 1 Beta	Direct	26.8
2	TNF	Tumor Necrosis Factor	Direct	25.19
3	IL6	Interleukin 6	Direct	24.62
4	MMP9	Matrix Metalloproteinase 9	Direct	18.65
5	VEGFA	Vascular Endothelial Growth Factor A	Direct	10.75
6	TP53	Tumor Protein P53	Direct	10.7
7	ESR1	Estrogen Receptor 1	Direct	9.17
8	JUN	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit	Direct	8.11
9	CCND1	Cyclin D1	Direct	7.5
10	MAPK8	Mitogen-Activated Protein Kinase 8	Direct	7.25
11	MAPK1	Mitogen-Activated Protein Kinase 1	Direct	7.15
12	IL2	Interleukin 2	Direct	7.13
13	CASP3	Caspase 3	Direct	6.36
14	NFKB1	Nuclear Factor Kappa B Subunit 1	Direct	6.35
15	FOS	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit	Direct	6.23
16	RELA	RELA Proto-Oncogene, NF-KB Subunit	Direct	5.93
17	STAT3	Signal Transducer And Activator Of Transcription 3	Direct	5.56
18	MAPK14	Mitogen-Activated Protein Kinase 14	Direct	5.53
19	NFKBIA	NFKB Inhibitor Alpha	Direct	5.33
20	CASP8	Caspase 8	Direct	5.26
21	PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	Indirect	5.18
22	HRAS	HRas Proto-Oncogene, GTPase	Direct	4.88
23	CDKN1A	Cyclin Dependent Kinase Inhibitor 1A	Direct	4.8
24	MYC	MYC Proto-Oncogene, BHLH Transcription Factor	Direct	4.55

The 34 genes were divided into two parts that are directly related and indirectly related to "OA". The score indicates the correlation between genes and the target phenotype

NO.	Symbol	Description	Direct/Indirect	Score
25	AKT1	AKT Serine/Threonine Kinase 1	Direct	4.5
26	ITGB3	Integrin Subunit Beta 3	Direct	4.27
27	BCL2	BCL2 Apoptosis Regulator	Direct	4.1
28	HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member 1	Direct	3.82
29	EP300	E1A Binding Protein P300	Direct	3.72
30	MAPK3	Mitogen-Activated Protein Kinase 3	Direct	3.22
31	APP	Amyloid Beta Precursor Protein	Direct	3.06
32	JAK2	Janus Kinase 2	Direct	3.06
33	EGFR	Epidermal Growth Factor Receptor	Direct	2.62
34	CREB1	CAMP Responsive Element Binding Protein 1	Direct	2.39
The 34 genes were divided into two parts that are directly related and indirectly related to "OA". The score indicates the correlation between genes and the target phenotype				

3.5 Clustering analysis in PPI network

Next, three dominant clustering subnetworks were obtained from 138 candidate targets by using MCODE algorithm in Cytoscape software (Fig. 5A). 15 core targets were included in cluster 1, mainly associated with TNF signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, Toll-like receptor signaling pathway. 10 core targets were involved in cluster 2, mainly involved in PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, TNF signaling pathway, HIF-1 signaling pathway and JAK-STAT signaling. 6 core targets were present in cluster 3, mainly related with MAPK signaling pathway, NOD-like receptor signaling pathway and PI3K-Akt signaling pathway (Fig. 5B).

3.6 KEGG enrichment analysis of core targets of scutellarin against OA

Enrichment analysis was performed by DAVID, as shown in Fig. 6. KEGG enrichment analysis indicated that 34 core targets of scutellarin against OA were mainly enriched in several kinds of pathways including TNF signaling pathway, PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and HIF-1 signaling pathway (Fig. 6).

3.6 Molecular docking of scutellarin and its potential targets

To further explain the inner relationship between scutellarin and its predicted targets related to OA, the key genes of NF- κ B signaling pathway including NFKBIA, NFKB1 and RELA, which were the key link of Hypoxia-inducible factor-1, NOD-like receptors and TNF- α signaling pathway, were selected to perform molecular docking. The results showed that there was better interaction between scutellarin and its potential targets, with the binding energy was -7.3 kcal/mol (NFKB1), -8.3 kcal/mol (NFKBIA), -7.2 kcal/mol (RELA), respectively (Fig. 7). In addition, the results showed Pi-cation, Pi-donor-hydrogen and Pi-alkyl were the main forms of interaction between scutellarin and NFKB1(1SVC) and NFKBIA(6Y1J), Pi-Pi T-shaped, Pi-alkyl and hydrogen bonding were the main forms of interaction between scutellarin and RELA(3QXY).

4. Discussion

OA, a biomechanical disease, which is estimated to be the fourth leading cause of disability by 2020[27, 28]. Although there are already several drugs for OA in clinical practice, including sodium hyaluronate, glucosamine, chondroitin sulphate, and NASIDs, most of them were not proved to truly prevent the progression of OA, and many side effects were appeared in application[29, 30], which may be due to the complicated pathological mechanism of OA, and the single-target drugs currently used in clinical practice could not fully prevent the progression of OA. Thus, our present study is trying to clarify the molecular mechanism of Scutellarin against OA by network pharmacology, aim to facilitate the development of Scutellarin, a safer agent derived natural plant, as a novel drug for OA, and promote development of OA therapy with natural plant derived agent mediated multi-target therapeutics. We collected information from public databases to predict potential targets of Scutellarin against OA, as well as to analyze the key signaling pathways and hub genes involved in the role of Scutellarin against OA. We also performed molecular docking to predict specific binding sites between Scutellarin and its predicted targets.

Firstly, we obtained the potential targets of Scutellarin against OA by using public database, and finally got 171 targets, which may be the important targets involved in the action of Scutellarin against OA. Then, we screened 34 core targets from 171 targets by degree score. Meanwhile, we performed cluster analysis and got three core clusters, which were principally linked to TNF signaling pathway, PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, HIF-1 signaling pathway, JAK-STAT signaling pathway and MAPK signaling pathway. In addition, we also found 34 core target genes were mainly enriched in TNF signaling pathway, PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and HIF-1 signaling pathway. These data indicated scutellarin might play the inhibitive effect on OA by regulating these signaling pathway. Previous reports showed scutellarin could regulate PI3K-Akt signaling pathway, MAPK signaling pathway, Wnt/ β -catenin signaling pathway and TLR4 signaling pathway, thus exerted the inhibitive action on OA progression. So, our data were partly in accordance with previous data. In addition, we also identified new signaling pathways including TNF signaling pathway, NOD-like receptor signaling pathway and HIF-1 signaling pathway, which were all the core signaling pathway in the pathogenesis of OA[31–36], thus may be involved in the inhibitive effect of Scutellarin on OA. Our data will be the supplement for the present studies on the molecular mechanisms of Scutellarin against OA. As we know, NF- κ B signaling

pathway was the key link of Hypoxia-inducible factor-1, NOD-like receptors and TNF- α induced OA progression [34, 36, 37]. NF- κ B signaling pathway was also the key target of the several anti-osteoarthritis compounds, including Scutellarin [11, 38, 39]. However, the action mode of Scutellarin on NF- κ B signaling pathway was still unknown. In our study, we found several key factors including NFKBIA, RELA and NFKB1 were involved in the 34 core targets of Scutellarin against OA. So, we next performed the molecular docking to clarify the action mode of scutellarin on NF- κ B signaling pathway. We found there are better docking between Scutellarin and three key factors of NF- κ B signaling pathway, indicating scutellarin may bind directly with NFKBIA, RELA and NFKB1, thus inhibit the activity of NF- κ B signaling pathway, finally play inhibitive effect on OA. Based on the literature and our data, several signaling pathways including PI3K-Akt signaling pathway, MAPK signaling pathway, Wnt/ β -catenin signaling pathway and TLR4 signaling pathway, TNF signaling pathway, NOD-like receptor signaling pathway and HIF-1 signaling pathway were possible signaling pathways involved in the activities of Scutellarin against OA. Among these, TNF signaling pathway, NOD-like receptor signaling pathway and HIF-1 signaling pathway were newly identified signaling pathway for clarification of the mechanisms of Scutellarin against OA. In addition, NFKBIA, RELA and NFKB1, the key factors of NF- κ B signaling pathway, may be the key targets associated with the activities of Scutellarin against OA by TNF signaling pathway, NOD-like receptor signaling pathway and HIF-1 signaling pathway.

Our study had several limitations. Firstly, the data set of the OA-related genes we used was based on existing research findings, which hinders the discovery of new targets for OA. Secondly, based on the topological analysis and key targets analysis of the constructed network, we only predicted the possible targets and signaling pathways associated with the activities of Scutellarin against OA. In the following study, we should verify the effect of Scutellarin on these key targets and signaling pathways by further biological experiments.

Overall, the current study found TNF signaling pathway, NOD-like receptor signaling pathway and HIF-1 signaling pathway were new possible signaling pathways and NFKBIA, RELA and NFKB1 were new possible targets associated with the activities of scutellarin against OA by a systematic network pharmacology and molecular docking. However, it is imperative that these targets should be thoroughly verified by *in vitro* and *in vivo* experiments.

Declarations

Authors' contributions

Mingli Sun and Xinan Zhang dominate the concept and carry out a comprehensive design, Shijia Guo is responsible for network pharmacology analysis and molecular docking. all authors participated in the analysis and interpretation of data and approved the final paper.

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Availability of data and materials

The additional data can be found in supplementary materials.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

There is no conflict of interest to declare.

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Figures

Figure 1

The workflow diagram for the prediction of potential targets of scutellarin against OA

Figure 4

The 34 core targets screened according to degree value were visualized by Cytoscape.

Figure 5

Cluster analysis and KEGG pathway enrichment of potential targets of scutellarin against OA. (A) Cluster analysis was done by MCODE algorithm based on the PPI networks of predicted targets, and the several core targets were clustered in different clusters. (B) The top 20 pathway enrichments of three clusters were analyzed by DAVID.

Figure 6

Pathway enrichment of 34 core genes. The top 20 pathway enrichments of 34 core genes were analyzed by DAVID and visualized by R software (A), and Cytoscape(B).

Figure 7

Molecular docking of scutellarin and its potential targets. The 3D and 2D presentation of molecular docking model between scutellarin and NFKB1(1SVC), NFKBIA(6Y1J) and RELA(3QXY). Scutellarin molecule was showed as a stick and residues were showed as lines in the binding sites. The hydrogen bonds, Pi-Pi T-shaped, Pi-Alkyl and Pi-cation were demarcated with dashed lines of different color, and the interaction distances were indicated next to the bonds.

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