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Original Research Article

A network pharmacology-based investigation of the mechanism involved in the anti-gastric cancer effect of *Oldenlandia diffusan*, a traditional Chinese medicine

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Abstract

Purpose: *To investigate the potential mechanism underlying the anti-gastric cancer (GC) effect of Oldenlandia diffusa using network pharmacology, and to provide scientific guidance for subsequent pharmacological and clinical translational studies.*
Methods: *The potential bioactive compounds in Oldenlandia diffusa and their related targets were obtained through TCMSP online platform. The GeneCards and MalaCards databases were used to search for GC-related disease targets. The targets shared by the two databases were entered into STRING protein interactions online database to obtain the interaction network of potential therapeutic targets. These were further screened for potential core targets through MCODE plugin. Cytoscape 3.2.1 software was used to construct the "component-target-disease" and PPI network, while GO and KEGG enrichment analyses were performed using DAVID v6.8 online software.*
Results: *Seven bioactive components and 180 drug targets were screened in Oldenlandia diffusa, out of which 167 targets were co-activated with GC, and 28 potential core targets were identified. The results of GO function enrichment analysis of the hub targets showed that they were related to gene transcription and expression, cytokine-mediated signaling pathway and inflammation response. The results of KEGG signaling pathway enrichment analysis showed that they were mainly associated with cancer signaling, IL-17-related signaling and TNF signaling pathways.*
Conclusion: *Oldenlandia diffusa exerts its therapeutic effect on GC through multi-component, multi-target and multi-signaling pathways. This finding provides novel evidence for the application of Oldenlandia diffusa in GC treatment.*

Keywords: *Oldenlandia diffusa, Gastric cancer, Network pharmacology, Bioinformatics, Mechanism of drug action*

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INTRODUCTION

Gastric cancer (GC) is a frequently seen malignant tumor of the digestive system in China. The incidence of GC is only second to those of lung cancer, breast cancer, colorectal cancer, and prostate cancer [1,2]. In spite of the rapid advances in methods used for clinical cancer therapy, mortality from GC ranks top three among all cancers, making it a major health

issue worldwide [2,3]. Therefore, there is need to evolve novel and efficient therapeutic approaches for GS. Several studies have demonstrated remarkable therapeutic effects of traditional Chinese medicines (TCM) on GC, thereby increasing research interest on identification of anticancer principles from Chinese herbal medicine [4,5].

Oldenlandia diffusa (OD), a member of Rubiaceae family, is a frequently-used TCM in clinical therapy for multiple malignancies such as leukemia, colorectal cancer, liver cancer, ovarian cancer, lung cancer and breast cancer [6-12]. Recent studies have confirmed that OD exerts antitumor effects through suppression of proliferation, angiogenesis and oxidation, as well as enhancement of apoptosis, and regulation of immune response [13-15]. For example, OD extracts induced apoptosis and inhibited growth of breast cancer cells in a mouse model via activation of the p53 signaling pathway and enhancement of macrophage function [16].

In view of the wide application of OD in tumor therapy and its good clinical effects, a systematic and comprehensive correlation amongst drugs, targets and diseases was performed using correlation analysis method of network pharmacology and bioinformatics so as to visualize drug action target network. Furthermore, the anti-GC potential of OD, and the potential mechanisms involved were investigated. This was aimed at providing new research strategies and methods for identifying the pharmacodynamic mechanisms, therapeutic outcomes and potential protein targets for GC.

EXPERIMENTAL

Identification of potential bioactive compounds and targets of OD

Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP) is a database and visualization platform based on the pharmacology framework of traditional Chinese herbal medicine system. It contains 499 Chinese herbal medicines registered in Chinese Pharmacopoeia, 29384 components, 3311 targets and 837 related diseases. The primary drug components and corresponding protein targets of *Oldenlandia diffusa* were searched using TCMSP database. The genes corresponding to the protein targets were searched using Uniprot online database, with the species identified as "human" to establish a data set for the subsequent analysis.

Prediction of potential targets of OD against GC

“Gastric cancer” was used as search item in the GeneCards and MalaCards online database. Data sets of disease-related targets were obtained by merging so as to eliminate repeated processing. Next, the overlapped items were extracted from targets of drug and disease as potential therapeutic targets of OD for GC.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

The GO and KEGG analyses of the potential targets were performed on DAVID Bioinformatics Resources 6.8 online enrichment analysis platform. Then, the enriched data were visualized using OmicShare cloud platform. Values of $p < 0.05$ were assumed as indicative of statistically significant differences.

Protein-protein interaction (PPI) network of the potential targets

Predicted proteins was analyzed to determine protein interaction and visualized using STRING Protein-Protein Interaction Networks Functional Enrichment Analysis Database (Version 11.0). The potential targets were searched using “multiple proteins” and “Homo sapiens” as restrictions, and confidence score at 0.700, and the results obtained were analyzed by CytoScape 3.2.1 software for visualization of the PPI network. Then, the core sub-network and modularization were constructed using MCODE plug-in (Degree Cutoff and K-core were set at 5).

RESULTS

Potential bioactive compounds from Oldenlandia diffusa and their potential targets

Seven (7) major bioactive compounds of *Oldenlandia diffusa* were retrieved from TCMSP database using “*Oldenlandia diffusa*” as keyword. Compounds with DL ≥ 0.18 and OB ≥ 30% were retained as bioactive components. Next, potential target genes of the bioactive compounds were predicted using TCMSP. The results presented 258 potential targets, which were further simplified to 180 items by excluding the repeated targets. Figure 1 shows the correlation network between the potential active compounds from *Oldenlandia diffusa* and the drug targets.

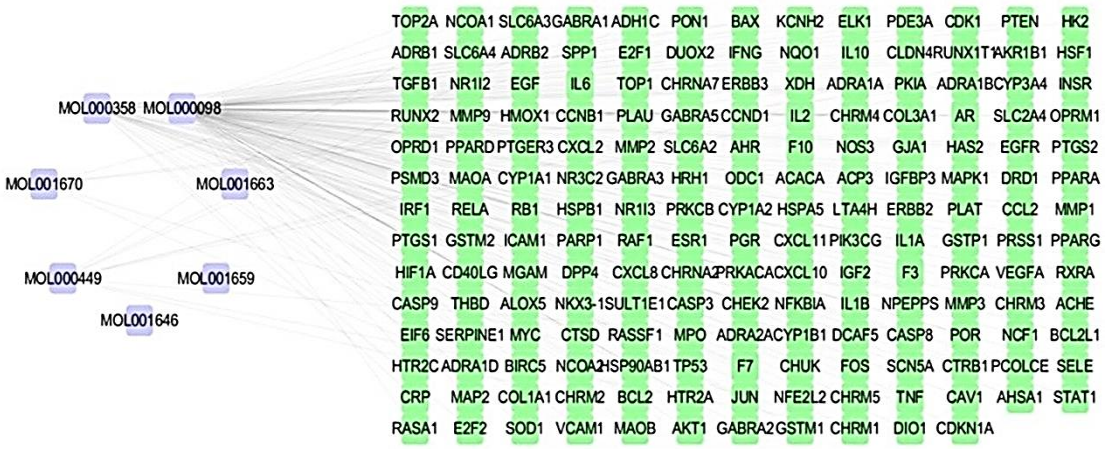

Figure 1: Screening of potential bioactive compounds from *Oldenlandia diffusa* and their potential targets. The correlation between the 7 potential bioactive compounds with potential targets was predicted using TCMSP

Potential targets of Oldenlandia diffusa against GC

In this study, 12309 targets and 516 targets that corelated with "gastric cancer" were retrieved from GeneCards and MalaCards online database, respectively. A total of 12309 potential targets for GC were obtained after excluding the overlapped ones. Common potential targets of gastric cancer and *Oldenlandia diffusa* were screened by the Venny 2.1.0 platform. The results showed 167 potential anti-GC genes of *Oldenlandia diffusa* (Figure 2). These were subjected to further analysis.

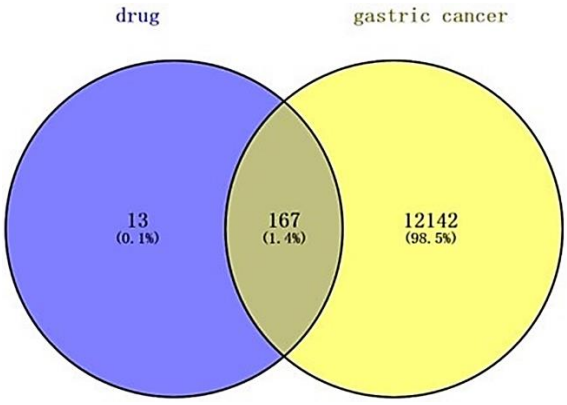

Figure 2: Potential targets involved in anti-GC effect of *Oldenlandia diffusa*. The overlapped targets of *Oldenlandia diffusa* and gastric cancer were plotted in Venn diagram

GO and KEGG enrichments of OD-targeted genes for GC

In the next step, GO and KEGG analyses were carried out to study *Oldenlandia diffusa*-regulated biological functions and related molecular mechanisms in GC. Results from GO analysis revealed that *Oldenlandia diffusa*-regulated biological processes were focused mainly on gene transcription, signal transduction, cell proliferation, and cell apoptosis (Figure 3 A). The possible cellular components modulated by *Oldenlandia diffusa* correlated mainly with the cytoplasm, nucleus, and plasma membrane (Figure 3 B). The molecular functional signals comprised binding ability of proteins and enzymes, and protein homodimerization activity (Figure 3 C). Results from KEGG analysis indicated that the *Oldenlandia diffusa*-targeted genes in GC were enriched in pathways associated with cancer, lipids and atherosclerosis, as well as chemical carcinogenesis-receptor activation, and PI3K-Akt signaling pathways (Figure 3 D).

PPI network of the screened potential targets

The 167 predicted targets of anti-GC effect of *Oldenlandia diffusa* were subsequently analyzed using STRING database. After excluding 15 unrelated proteins, the interaction analysis of the 152 potential targets were visualized using Cytoscape 3.2.1 software. The resultant PPI network contained 152 nodes and 2028 lines (Figure 4). The color and of nodes reveal the "degree" of each node, with the bigger sizes and darker colors indicating higher degrees. The lines between nodes indicated the interrelationships of the predicted targets.

Subsequently, the core network was extracted using MCODE plug-in, resulting in a sub-network containing 28 nodes and 316 lines with a MCODE score of 11.704 (Figure 5 and Table 1). Moreover, GO and KEGG analyses of the screened 28 core genes were performed.

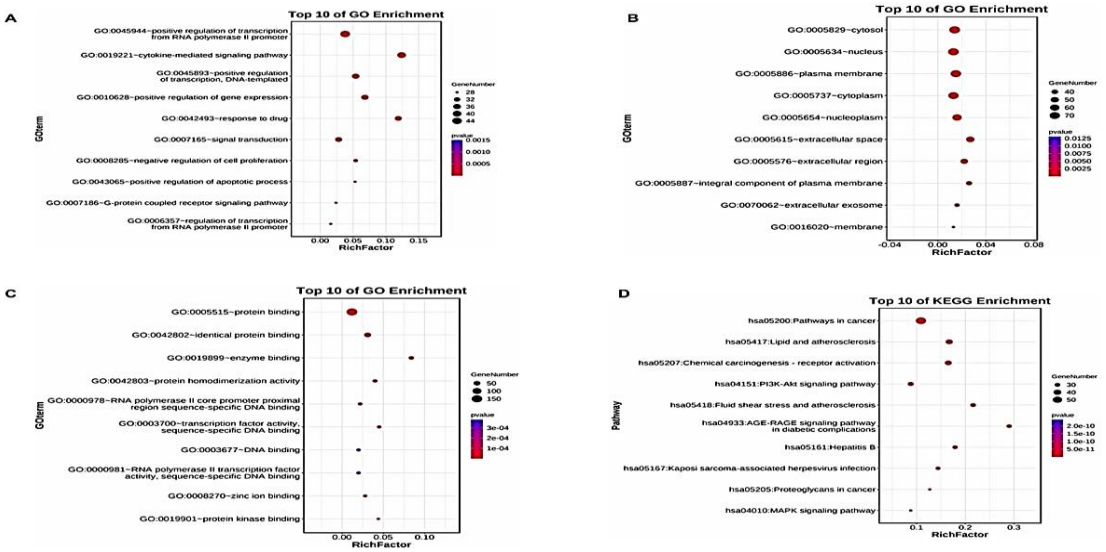

Figure 3: Functional enrichment of *Oldenlandia diffusa*-targeted genes for GC. (A) Top 10 enriched biological processes. (B) Top 10 enriched cellular components. (C) Top 10 enriched biological processes. (B) Top 10 enriched molecular functional signals. (D) Top 10 enriched KEGG signaling pathways

Results from Figure 6 showed that the 28 potential targets were correlated with biological processes such as gene transcription and expression, cytokine-mediated signaling pathway, and inflammatory response (Figure 6 A), as well as cellular components such as extracellular space, transcription factor AP1 complex, and RNA polymerase II transcription factor complex (Figure 6 B). Moreover, the targets were correlated with molecular functional signals involving protein binding, transcription factor binding, and enzyme binding (Figure 6 C). The core genes were mainly enriched in signaling pathways such as pathways in cancer, IL-17 signaling, and TNF signaling (Figure 6 D).

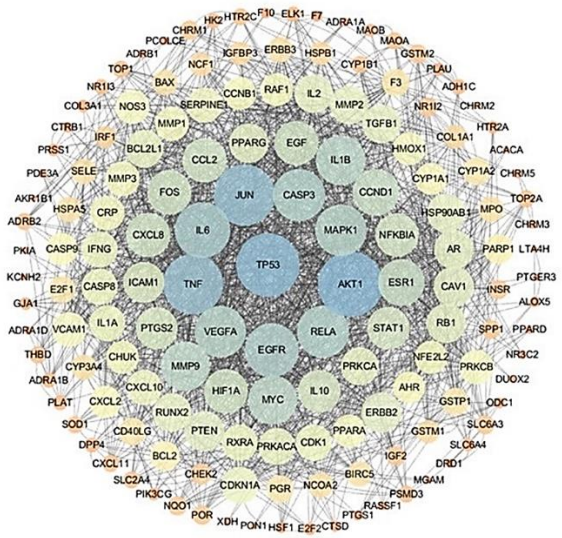

Figure 4: PPI network of the 152 potential targets. The PPI network of the screened 152 potential targets was established using Cytoscape 3.2.1 software

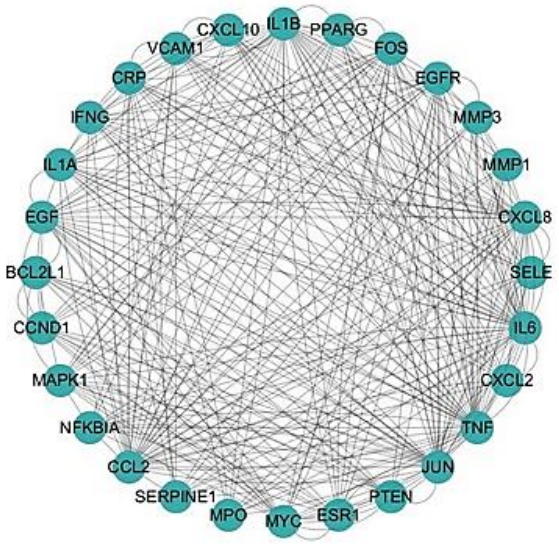

Figure 5: The core sub-network of the predicted targets of *Oldenlandia diffusa* against GC. The core sub-network was established using MCODE plug-in

Table 1: Hub targets in GC screened using MCODE

S/no.	Core target	MCODE score
1	IL6	60.71001628
2	IL1B	46.38077701
3	EGFR	45.3068485
4	FOS	29.4339088
5	IL1A	25.17164021
6	IFNG	24.87987711
7	CRP	24.23930795
8	CXCL10	23.13488433
9	BCL2L1	22.40503322
10	VCAM1	21.23006135

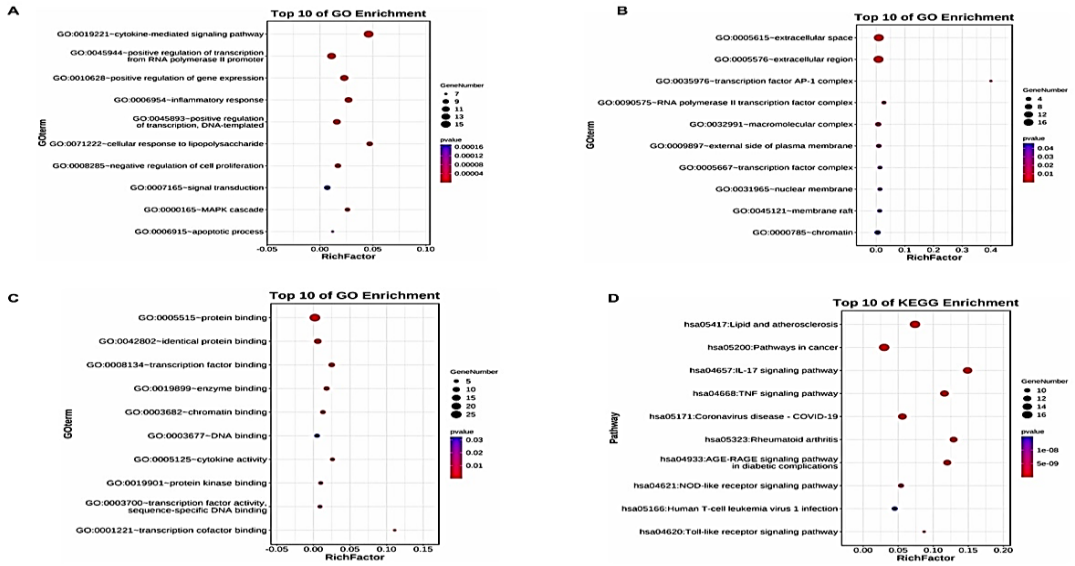

Figure 6: Functional enrichment of 28 core targets from the core sub-network. (A) Top 10 enriched biological processes. (B) Top 10 enriched cellular components. (C) Top 10 enriched biological processes. (B) Top 10 enriched molecular functional signals. (D) Top 10 enriched KEGG signaling pathways

Interaction network of bioactive compounds and key targeted genes in GC

The correlation amongst the predicted bioactive compounds of *Oldenlandia diffusa* and the 28 potential targets obtained from above sub-network was determined. Results from Figure 7 indicated that the constructed interaction network contains 31 nodes and 29 lines. The circular nodes represent the bioactive compounds of *Oldenlandia diffusa*; the rhombus nodes represent potential targets in GC, while the lines indicate correlation amongst nodes.

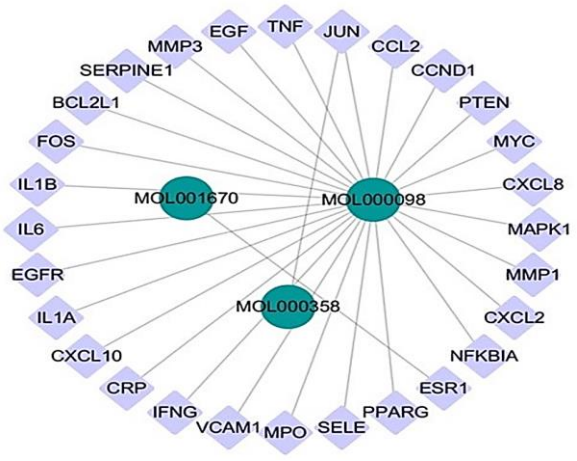

Figure 7: The interaction network between bioactive compounds and key targeted genes in GC. The circular nodes represent the bioactive compounds of *Oldenlandia diffusa*, while the rhombus nodes represent potential targets in GC

The more connecting lines between nodes indicate the more important roles they play in the network. Thus, the corresponding components or targets may be key components or promising therapeutic targets of *Oldenlandia diffusa* for GC treatment. Moreover, the constructed network suggests that among the bioactive compounds of *Oldenlandia diffusa*, MOL000098 (quercetin) and MOL000358 (β-sitosterol) are the most important monomer components in terms of effect on GC.

DISCUSSION

In recent years, network pharmacology has become a popular and efficient method used for analyzing the connection amongst the bioactive compounds of TCM, targets and diseases, thereby elucidating the pharmacological properties of medicinal plants using multi-level network model, as well as identifying the bioactive components and their potential mechanisms [17-20]. In this work, network pharmacology was adopted to preliminarily explore the key components, core targets and related biological signaling pathways involved in the anti-GC potential of the herbal medicine *Oldenlandia diffusa*.

In the analysis of interaction network, quercetin and β-sitosterol were identified as the major potential bioactive components involved in the anti-GC function of *Oldenlandia diffusa*. Studies have demonstrated the good antitumor effects of quercetin and β -sitosterol on several cancers. For example, Garcia *et al.* [21] reported that quercetin in foods significantly decreased the incidence of GC. β-Sitosterol has also been shown to suppress the growth of breast cancer, colon cancer, as well as prostate cancer [22-24].

The results from PPI network analysis revealed the core targets involved in the anti-GC potential of *Oldenlandia diffusa.* These targets were IL-6, IL-1β, EGFR, proto-oncogene C-fos (Fos) and IL-1α, most of which are inflammatory factors. In normal healthy state, these cytokines maintain intracellular homeostasis, whereas upon inflammation and impairment of homeostasis, mutations occur in the cytokines, leading to pathological and physiological changes in the stomach, which further promote or prevent GC [25,26]. IL-1β exhibits various biological functions such as mediating inflammatory response, inducing epigenetic changes in genes, promoting angiogenesis, and inhibiting gastric acid production; gastric acid secretion is a major cause of chronic atrophic gastritis [27-29]. Interleukin-1β (IL-1β) is secreted by multiple cell types such as epithelial cells, tumor cells, macrophages and monocytes, and it mediates inflammatory response and enhances the occurrence, invasion, and immunosuppression of tumors [30,31].

Results from GO analysis demonstrated that the 167 potential anti-GC targets of *Oldenlandia diffusa* were involved in gene transcription, gene expression, signal transduction, cell proliferation, and cell apoptosis. Further biological process analysis of 28 potential target genes in the core sub-network of PPI demonstrated that these targets were correlated with gene transcription and expression, cytokine-mediated signaling pathway, inflammatory response, cell proliferation and apoptosis, and MAPK cascade. The KEGG enrichment analysis of the 167 potential targets involved in the anti-GC effect of *Oldenlandia diffusa* revealed that these targets may participate in cancer-related signaling pathway, activation of chemical carcinogenic factor receptors, PI3K-Akt pathway, and MAPK pathway. Furthermore, the screened 28 potential targets in the core sub-network of PPI were closely associated with cancer-related signaling pathways, TNF pathway, and IL-17 pathway.

CONCLUSION

Taken together, the results obtained in this study suggest that the mechanism involved in the anti-GC effects of *Oldenlandia diffusa* may be correlated with pathological processes such as resistance to cell apoptosis, abnormal cell cycle, cell differentiation and proliferation, cell migration and invasion, and angiogenesis, and their associated signaling pathways. These findings provide a scientific basis for further clinical translational research on the anticancer potential of *Oldenlandia diffusa* with respect to GC.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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