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Exploration on the Mechanism of Action of Huangqin and Gegen in the Treatment of Non-Alcoholic Fatty Liver Disease Based on Network Pharmacology

Zhou Changjing[1], Lu Bo[2,*]

[1]Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712046, China
[2]Shaanxi Provincial Hospital of Chinese Medicine, Xi'an, Shaanxi, 710003, China
[*]Corresponding author

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Abstract: In order to investigate the mechanism of action of Scutellaria baicalensis-gem root medicines in the treatment of non-alcoholic fatty liver disease (NAFLD) using a network pharmacology approach. We used the TCM Systematic Pharmacology Database and Analysis Platform (TCMSP) and Uniprot database to screen the active ingredients and targets of Scutellaria baicalensis and Pueraria lobata. The GeneCard and OMIM databases were accessed to search for disease-related targets; the anti-NASH targets of GGQLT were obtained by using the Wayne Diagram online software; the egg-autoplasmic-egg-autoplasmic interaction (EAEI) network was created by using Cytoscape 3.7.2; and the Metascape online software was used to perform the gene ontology (GO) enrichment and Kyoto Gene and Genome Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis. Resultly, we obtained 19 components of Scutellaria baicalensis and Pueraria lobata with 540 targets. 3,610 NASH targets and 57 Scutellaria baicalensis-kudzu drug-antagonistic NAFLD sites were obtained. Visualization analysis showed that the top 3 scutellaria baicalensis-kudzu drug against NAFLD targets were estrogen receptor 1 (ESR1), B lymphoblastoma-2 gene (BCL2), and mitogen-activated protein kinase 8 (MAPK8). 179 entries were obtained by GO enrichment analysis, and 145 signaling pathways were obtained by KEGG signaling pathway analysis. In conclusion, in this study, we obtained the chemical constituents and targets of Scutellaria baicalensis-Geranium medicinal pairs through network pharmacology, screened the key constituents and key targets of Scutellaria baicalensis-Geranium against NAFLD, and analyzed the mechanism of action of Scutellaria baicalensis-Geranium medicinal pairs against pitted NAFLD, in order to provide an opportunity for Scutellaria baicalensis- Pueraria Mirifica drug on the treatment of NASHFLD to provide more theories and bases, and at the same time to provide a certain theoretical basis for further research on Scutellaria baicalensis - Pueraria Mirifica drug on the treatment of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a liver lesion in which excessive fat accumulates in

the liver but is not caused by alcohol consumption [1], and is closely related to genetics, insulin resistance and disorders of glucose and lipid metabolism. Epidemiological surveys show that the global prevalence of NAFLD is as high as 25.24% as of 2022 [2], and NAFLD can develop into cirrhosis and hepatocellular carcinoma if not intervened in a timely manner, seriously jeopardizing the quality of patients' survival. A prospective study found that the incidence of NAFLD and hepatocellular carcinoma in diabetic patients is twice as high as that in non-diabetic patients [3], and on the other hand, NAFLD can aggravate insulin resistance (IR) and affect glucose and lipid metabolism, resulting in a significant increase in the incidence of cardiovascular events in T2DM patients [4]. In this study, we screened the active components and potential targets of NAFLD based on network pharmacology, predicted the potential targets and mechanisms of action of Huangqi - Ge'an drug pairs for the treatment of NAFLD, analyzed the biological functions and signaling pathways involved in the key proteins, and provided theoretical bases for the pharmacological mechanisms of Huangqi -Ge'an drug pairs for the treatment of NAFLD [5]. The study was conducted to analyze the potential targets and mechanisms of the key proteins.

2. Materials and methods

2.1. Screening of active ingredients and targets of action and disease targets of Chinese medicines

The potential active ingredients and targets of Scutellaria baicalensis and Pueraria lobata were searched in TCMSP database (https://tcmspw.com/index.com/index.php), and bioavailability (OB) ≥30% and drug-like properties (DL) ≥0.18 were set as the screening conditions to screen the active ingredients and targets. Active ingredient and target point of action. Bioavailability is an important parameter for evaluating the efficacy and safety of a drug, and refers to the degree and speed of absorption of a drug into the body circulation after oral administration. Drug-like properties refer to the physical properties of a drug whose structure and pharmacological effects are similar or identical to those of most existing drugs, and are the key parameters for the screening of TCM components [6].

The active ingredients were screened in TCMSP database and imported into Uniprot database (https://www.uniprot.org/), the target gene screening species was limited to human and then the gene name was modified to the official name after deleting the active ingredient without target, and the ingredient-target was obtained by using perl software. The ingredient-target database was obtained by running perl software. We logged into GeneCard database (https://www.genecards.org) and OMIM database (https://www.omim.org) [7], and searched for disease-related components using the keywords "non-alcoholic fatty liver disease" and "non-alcoholic fatty liver disease". "Non-alcoholic fatty liver disease" was used as the keyword to search for disease-related targets, and the duplicate targets were eliminated to construct the NAFLD-related target dataset. Matching the drug and disease targets, we constructed a dataset of potential targets of Scutellaria baicalensis and Pueraria lobata for the treatment of NAFLD, and drew a Wayne diagram using online Venny.

2.2. Chinese medicine components and disease interaction network

The full names of the genes of the drug action targets obtained from TCMSP were transformed into genesymbol - a unique abbreviation of the gene name - through the UniProt database. The intersection of the active ingredient-target database of Scutellaria baicalensis and Pueraria lobata with the NAFLD gene database was used to obtain the genes related to the effects of Scutellaria baicalensis and Pueraria lobata on NAFLD. -Cytoscape mapped the regulatory network of "active ingredients and target genes of traditional Chinese medicine".

2.3. Protein Interaction Network (PPI)

Through the String website, we selected human species, set confidence level=0.7, hid the dropout nodes for screening. And then we outputed the TSV file, imported the data into Cytoscape, and optimized the data using the CytoNCA plug-in. The six parameters of mediator centrality, proximity centrality, point degree centrality, eigenvector centrality, local average connectivity were selected. And we scored and filtered network centrality in the plugin, while retaining genes with scores greater than the median. The genes retained after filtering twice were defined as the core genes of the PPI network.

2.4. GO bioprocess enrichment analysis and KEGG pathway enrichment analysis

GO and KEGG database analyses were performed using Metascape online software to enrich the pathway information of genes related to Scutellaria baicalensis-gem root action in NAFLD, with $P < 0.05$ as the criterion for significant enrichment.

3. Results

3.1. Active ingredients and targets of action

The active ingredients of the TCMSP-predicted Scutellaria baicalensis-gem root pairs were screened under the conditions of OB ≥ 30% and DL ≥ 0.18, and the components without target proteins were removed, and finally 16 active ingredients of Scutellaria baicalensis and 3 of Pueraria lobata were identified. A total of 560 target genes corresponding to the Scutellaria baicalensis-Pueraria lobata pairs were obtained by converting gene symbols to full gene names through the UniProt database and eliminating duplicate values.

3.2. Prediction of human NAFLD target genes

A total of 3,610 disease target genes were obtained by combining the 560 potential action targets screened by Scutellaria baicalensis-kudzu root with the human NAFLD-related genes obtained from the summarized GeneCards and OMIM databases and de-emphasizing them.

3.3. Screening of drug-disease intersecting target genes

Online mapping of Scutellaria baicalensis and Pueraria lobata active ingredient targets and NAFLD disease intersecting targets was carried out by R software, and 57 intersecting targets were obtained (Figure 1).

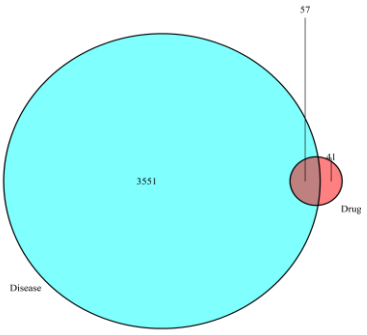

Figure 1: Veen plot of Scutellaria baicalensis-grape root pairs of targets associated with nonalcoholic fatty liver disease

3.4. Construction of the "active ingredient-target" network

The active ingredients and targets of Scutellaria baicalensis and Pueraria lobata were imported into Cytoscape software to construct the "active ingredient-target" network diagram (Figure 2), which has 118 nodes and 265 edges, with rhombuses representing the drugs, pink ovals representing the active ingredients, and inverted triangles representing the active ingredients corresponding to the active ingredients. The rhombus represents the drug, the pink oval represents the active ingredient, the inverted triangle represents the target corresponding to the active ingredient, and the purple oval is the active ingredient common to traditional Chinese medicine.

3.5. Core target screening

The 57 intersecting genes were analyzed by PPI using the STRING platform, and the free nodes were removed. The output TSV files were imported into Cytoscape software, resulting in 57 nodes and 80 edges, 42 key targets, and a PPI network map (Figure 3), among which estrogen receptor 1 (ESR1), B lymphoblastoma-2 gene (BCL2), and mitogen-activated protein kinase 8 (MAPK8) had large values, which were predicted to interact with other proteins in the PPI network. The high degree of ESR1 was predicted to interact with other proteins in the PPI network. Among them, ESR1 had the highest degree value, suggesting that it may be a more important potential target of Scutellaria baicalensis-kudzu for the treatment of NAFLD.

3.6. GO biological process enrichment analysis and KEGG pathway enrichment analysis

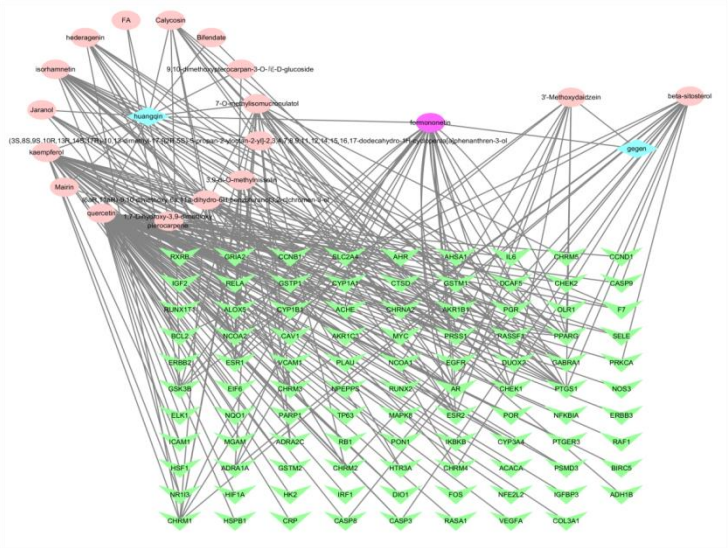

Figure 2: Drug active ingredient-target network diagram

In the analysis results, there were 179 items of GO analysis: 75, 75, 29 items of molecular function (MF)(Figure 4), biological process (BP)(Figure 5) and cellular component (CC)(Figure 6) respectively. According to the descending order of Count value and P<0.05, there were 16, 16, 11 entries each, and the bar charts were plotted by using the microbiology platform in Figure 4, which were mainly involved in the regulation of gene expression, cell proliferation and apoptosis, inflammation, etc.; in the cytoplasm, nucleus, polymer complexes, exosomes, etc.; and in the molecular functions, such as protein binding, DNA binding, etc. The KEGG results were 145 entries (Figure7). The 145 KEGG results were sorted in descending order according to the Count value with P<0.05, and the top 20 items were screened out and plotted as bar charts by using the microbiology

platform, see Figure 4. The irrelevant signaling pathways, such as pancreatic cancer, hepatitis and prostate cancer, were excluded, and the related signaling pathways, such as MAPK8, ESR1, FOS, HIF1A, MYC, PPARG, and RELA, were retained.

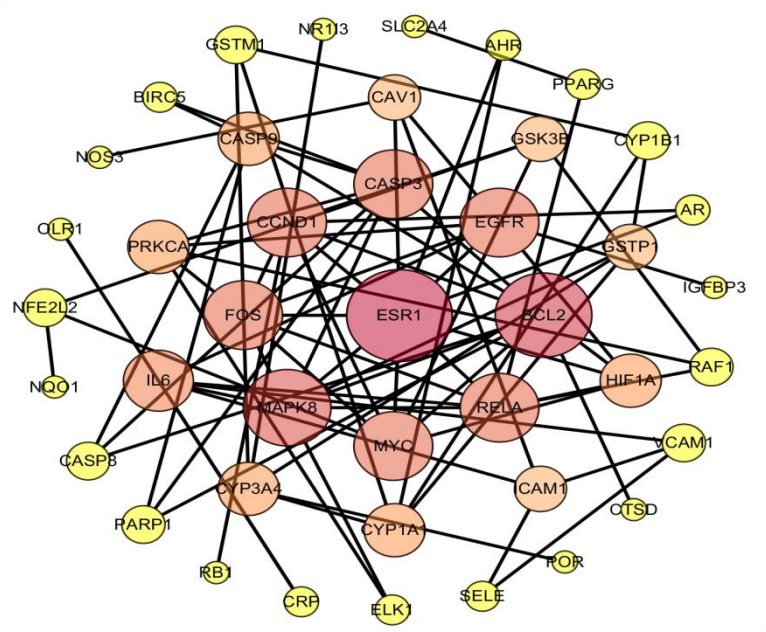

Figure 3: PPI network diagram of 42 key target proteins

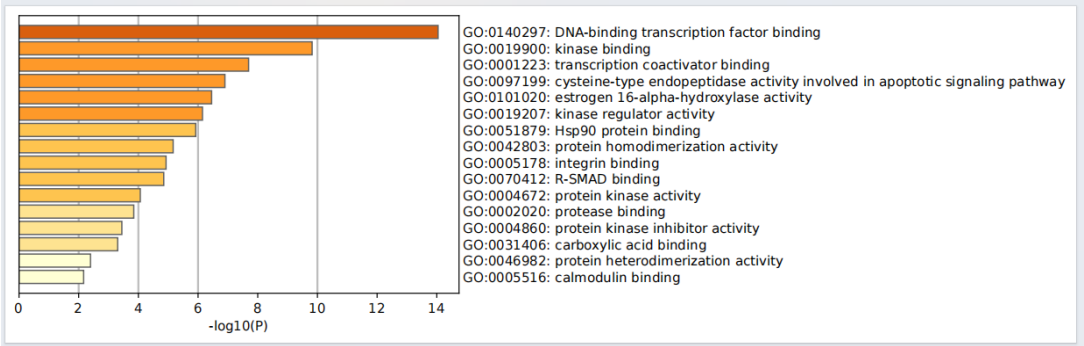

Figure 4: GO (Molecular Function) enrichment analysis plot

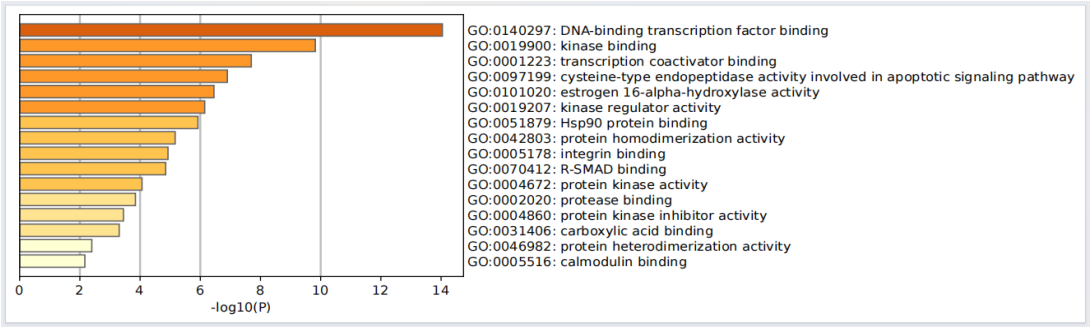

Figure 5: GO (Biological Process) enrichment analysis plot

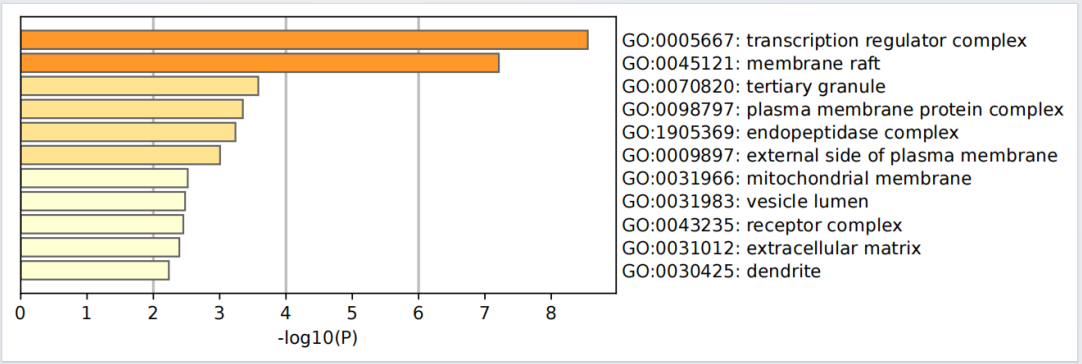

Figure 6: GO Cellular Component) enrichment analysis plot

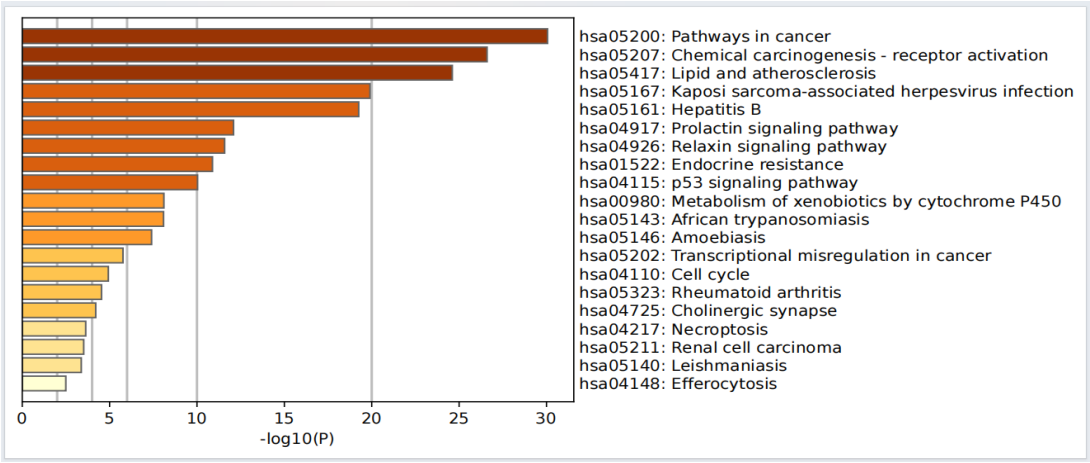

Figure 7: KEGG enrichment analysis plot

4. Discussion

Studies have shown that Scutellaria baicalensis has hepatoprotective effects [8], and in the field of fatty liver, it has been shown that baicalin can significantly reduce plasma cholesterol levels, free fatty acid content and hepatic fat deposition in rats fed a high-fat diet [9]. Through the network pharmacology approach, 19 potential active ingredients and 560 potential targets were screened for the scutellariae baicalensis-graecum drug pair. Among them, mangostin is the common active ingredient of the pair of Chinese medicines, which can be aligned with multiple targets of action. Stinging mangostin is the main component of red axillaris, which belongs to the isoflavonoids, polyphenol nonsteroidal phytochemicals with estrogenic activity, and it is commonly found in a variety of plants such as Radix Astragali, Chickweed and so on, and at the same time it has the effects of antitumor, antioxidant, and anti-inflammatory [10]. Meanwhile, different active ingredients of Scutellaria baicalensis and Pueraria lobata can act on the same targets, suggesting that the Scutellaria baicalensis-Pueraria lobata drug pair produces therapeutic effects through the synergistic effect of multiple components. In this study, the PPT protein interaction network was screened twice, and 42 core nodes were finally retained, suggesting that the interactions between these target-expressed proteins may be the key to prompting Scutellaria baicalensis-Gerbera root medicine in the treatment of NAFLD. After GO biofunction enrichment analysis and KEGG enrichment analysis, we analyzed the top 3 biofunctions and signaling pathways significantly affected by Scutellaria baicalensis-kudzu drug, and found that the relevant biofunctions were focused on the response to cell proliferation, apoptosis, inflammatory response, oxidative stress counterattack, adipose differentiation and metabolism, and hypoxic stress response, etc.; among the signaling pathways related to NAFLD, they

included the MAPK signaling pathway, estrogen signaling pathway, Ras/MAPK signaling pathway, hypoxia response, PPAR signaling pathway, and NF-kB signaling pathway. In summary, 19 potential active ingredients such as β-glutosterol, 3'-methoxy soybean flavonoids, kaempferol, jalol, and ivy saponin elements contained in the Scutellaria baicalensis-kudzu root pairs may act on ESR1, AR, PPARG, GSK3B, PRSS1, CHRM3, BCL2, CASP9, and other 560 potential targets, through the interactions between the expressed proteins of these target genes, activate MAPK8 signaling pathway, estrogen signaling pathway, Ras/MAPK signaling pathway, hypoxia response, PPAR signaling pathway, NF-kB signaling pathway, etc., so as to regulate the cell biological behaviors of cell proliferation, apoptosis, inflammatory response, oxidative stress counterattack, fat differentiation and metabolism, hypoxic stress and so on, that play a role in NAFLD therapy. Scutellaria baicalensis-kudzu drug pair has multi-component, multi-target, and multi-pathway effects in the treatment of NAFLD, and the MAPK signaling pathway may be one of the key pathways of Scutellaria baicalensis-kudzu drug pair in prolonging the survival of patients with advanced intestinal cancer [11].

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