Research Article

Effects of Reducing the South and Reinforcing the North Method on Inflammatory Injury Induced by Hyperlipidemia

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Inflammation is the pathophysiological basis of hyperlipidemia-related disease (HRD). Reducing the south and reinforcing the north method (RSRN) has a positive effect on HRD. However, the pharmacological mechanisms of RSRN are still unclear in the treatment of HRD. We obtained RSRN compounds from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) and identified potential targets of these compounds through target fishing based on the TCMSP databases. Next, we identified the HRD targets by using multiple databases. Then, the overlapping genes between the RSRN potential targets and the HRD targets were used to establish a protein-protein interaction (PPI) network, and we further analyzed their interactions and identified the major hub genes in this network. Subsequently, the Metascape database was utilized to conduct the enrichment of Gene Ontology biological processes (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. A total of 187 potential active components and 106 related core targets were obtained and identified overall. After the Metascape enrichment analysis, a total of 148 KEGG pathways were screened, which were mainly associated with AGE-RAGE signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway, and NF-kappa B signaling pathway. Furthermore, 34 hub genes, such as AKT1, NF-κBp65(RELA), IκBα(CHUK), MAPK8, and MAPK14, CCND1, were considered potential therapeutic targets. Furthermore, evaluations of protein levels of NF-κBp65, IκBα, TNF-α, IL-1β, and IL-6 were performed for experimental validation. RSRN can reduce the expression of NF-κBp65 protein, increase the level of IκBα protein, and reduce the protein levels of TNF-α, IL-1β, and IL-6 in ovariectomized rats. The results indicate that the mechanism of RSRN against inflammation may be related to AKT1, NF-κBp65, IκBα, MAPK8, and MAPK14 signaling pathways.

1. Introduction

The decreased ovarian function and estrogen levels in menopausal women contributes to the increase in the prevalence of dyslipidemia, osteoporosis, and urinary tract infection, of which hyperlipidemia is the most insidious [1]. Previous studies have reported that hyperlipidemia could lead to atherosclerosis (AS), coronary heart disease (CHD), and Alzheimer’s disease [2, 3]. There should be necessary interventions to be done for preventing the hyperlipidemia related diseases (HRD), thus to reduce the incidence rate and mortality of AS and CHD in middle-aged and elderly women.

Traditional Chinese medicine (TCM) believes that the kidney is the congenital life basis. When women are in the menopausal period, kidney-Qi declines; Tiangui will be exhausted; Chong and Ren become deficient; essence and blood become insufficient; all these conditions can lead to imbalance between Yin and Yang and Zang-fu organs disorders, resulting in menopausal syndromes and hyperlipidemia-related diseases [4]. The pathogenesis of HRD belongs to the syndrome of defciency of origin and excess of standard, while kidney Yin defciency is the foundation, and heart fire is excessive, and essence defciency and blood stasis is the standard [5]. In clinic, method of nourishing kidney and clearing heart can effectively alleviate and control these syndromes. Therefore, reducing the south (clearing heart) and reinforcing the north (nourishing kidney essence) may be the foundation of the HRD treatment. Reducing the south and reinforcing the north formula (RSRN) consist of eight
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2.1. Identification of Main Active Components and Related Herbs: The threshold of OB at parameters of the oral bioavailability (OB); we set the -X_he active components were further identified using the TCMSP database (http://lsp.nwu.edu.cn/tcmsp.php) [11]. Of the constituent data of RSRN were obtained from the database of traditional Chinese medicine (TCMID), http://119.3.41.228:8000/TCMID/prescription search/). -X_hen all database of traditional Chinese medicine (TCMID), http://bidd.nus.edu.sg/group/cjttd/), DrugBank database (https://www.durgbank.ca/), OMIM database (Online Mendelian Inheritance in Man (https://www.omim.org/), and then the acquired disease targets were evaluated by functional enrichment analyses. GO analysis was performed in three categories, namely biological processes (BP), cellular component (CC), and molecular function (MF), and the KEGG signaling pathway analysis was performed with R software using the Bioconductor package. The KEGG pathway was introduced into Cytoscape 3.6.0. According to the degree value, the enrichment degree of pathway and gene was displayed, and the network diagram was drawn.

2.2. Disease Targets Identification by Multiple Databases. “Hyperlipidemia,” “atherosclerosis,” and “Alzheimer’s disease” were selected as the key words for the retrieval of disease targets from the GeneCards database (https://www.genecards.org/), TTD database (Therapeutic Target Database, http://bidd.nus.edu.sg/group/cjtd/), DrugBank database (https://www.durgbank.ca/), OMIM database (Online Mendelian Inheritance in Man (https://www.omim.org/), and then the acquired disease targets were intersected by FunRich3.1.3 software. GSE57691 was screened and downloaded from the GEO database (gene expression omnibus, https://www.ncbi.nlm.nih.gov/geo/) with “hyperlipidemia” and “atherosclerosis” as the key words, including 58 cases of disease group (49 cases of abdominal aortic aneurysm and 9 cases of aortic occlusive disease) and 10 cases of normal control group. We merged the above results and deleted the duplicate genes to obtain the final disease target genes by R 4.0.2 software.

2.3. Construction and Analysis of Drug-Disease Target Network. Based on previous steps, drug-disease crossover genes were screened with R software using the Venn Diagram package. The String 11.0 database (http://string-db.org/) was used to analyze the intersecting protein-protein interactions (PPIs) and then Cytoscape3.6.0 was used to determine the drug-disease target network. The “centiscape” plug-in was used to calculate the degree of freedom of drug-disease target. The higher the “degree” value, the greater the probability of playing the main function [13]. Using the Bisogenet and CytoNCA plug-ins of Cytoscape software (version 3.6.0), we set the parameters of degree centrality (DC > 61) and the topology intermediateness (BC > 600) and constructed the core target gene topology network; furthermore, we screened out the important candidate genes.

2.4. GO and KEGG Pathway Analysis. The screened drug-disease targets were evaluated by functional enrichment analyses. GO analysis was performed in three categories, namely biological processes (BP), cellular component (CC), and molecular function (MF), and the KEGG signaling pathway analysis was performed with R software using the Bioconductor package. The KEGG pathway was introduced into Cytoscape 3.6.0. According to the degree value, the enrichment degree of pathway and gene was displayed, and the network diagram was drawn.

2.5. Herbal Preparation. We prepared RSRN using the following constituents: 30 g of Curculigo orchioides Gaertn (Xian Mao), 30 g of Epimedium Folium (Yin yang huo), 15 g of Morinda officinalis Radix (Ba ji tian), 15 g of Angelicae Sinensis Radix (Dang gui), 12 g of Phellodendron chinense Cortex (Huang bo), 10 g of Anemarrhenae Rhizoma (Zhi mu), 12 g of Salviae Miltiorrhizae Radix et Rhizoma (Dan shen), and 6 g of Coptidis Rhizoma (Huang lian). We obtained all herbs from Longhua Hospital Shanghai University of TCM, China. The herbs were mixed, soaked in water for 0.5 h, and decocted for 1 h in 5% v/w distilled H2O at 100°C. Subsequently, the filtrate was collected, and the residue decocted for another hour with 5% v/w distilled water. Next, the filtrate was concentrated (RE-3000B, Ya-rong Biochemical Instrument Shanghai Co., Ltd) and lyophilized (LGJ-10D, Four-ring Science Instrument Plant Beijing Co., Ltd), and the resulting RSRN powder was kept at −20°C until use. HPLC was used to determine the components in RSRN, and determination of curculigoside, Dihydrotanshinione I, Icariisid I, Magiferin, Sarosapogenin, Jatrorrhizine in RSRN is shown in Figure 2.
2.6. Animals and Administration. We obtained 40 healthy eight-week-old female Sprague-Dawley (SD) rats (mean weight: 200 ± 20 g) from Shanghai Slack Laboratory Animals Co., Ltd. [license number: SCXK (Hu) 2012–0002]. All the rats were housed in an air-conditioned room with 12 h light-dark cycles at a constant temperature (22–26°C) and humidity (50% ± 10%). Further, the rats were provided with rodent chow and tap water ad libitum. The animal model of climacteric atherosclerosis was established by bilateral ovariectomy and high-fat diet [14]. After acclimation for one week, the rats underwent either ovariectomy (n = 30) or sham operation (n = 10) under anesthesia using an intraperitoneal injection of 30 mg/kg pentobarbital sodium. Nine days after surgery, we randomly divided 30 OVX rats into the following three groups: OVX (high-fat emulsion [15] with an equal volume of ddH2O; 1 mL/100 g/day), RSRN (high-fat emulsion with RSRN; 5.46 g/kg/day), and EV groups (high-fat emulsion with estrogen valerate; 0.1 mg/kg/day). The sham-operated rats (SHAM group) received a normal diet with an equal volume of ddH2O; 1 mL/100 g/day. During the experimental period, the rats underwent weekly weight measurements. All procedures were approved by the Department of Laboratory Animal Science Longhua Hospital Shanghai University of TCM. The animal welfare and experimental procedures were conducted in strict accordance with the guidelines for the care and use of experimental animals and the ethics of Shanghai University of TCM. For the next six weeks after the last administration, the rats were food- and water deprived for 12 h. The rats were anaesthetized by diethyl ether inhalation. The brain and the blood were taken for further use.

2.7. Immunohistochemistry Analysis. Immunohistochemistry procedure was performed as previously reported protocol [16]. The sections were incubated with the primary antibody for NF-κB p65 overnight at 4°C (#8242; 1:100, Cell Signalling Technology, MA, USA). After washing, the sections were incubated with the secondary antibody (#MR-R100; MR Biotech, Shanghai, China) for 1 h and then stained with 3,3′-diaminobenzidine (DAB) (Biyuntian Institute of Biotechnology, Jiangsu, China). Signals were visualized by light microscopic observation. The results were analyzed by using Image J software version1.50i (National Institute of Health, USA). The investigators used the software to measure the ratio of positive area (A%).

2.8. Western Blotting Analysis. The protein samples were separated by 10% SDS-PAGE transferred to PVDF membrane. The membrane was blocked with 1% BSA in TBST for 30 min, the primary antibodies including NF-κBp65 (#8242; 1:1000; Cell Signalling Technology, MA, USA), IκBα (#4812; 1:1000; Cell Signalling Technology, MA, USA), TNF-α (#sc-52746; 1:1000; Santa Cruz Biotechnology, USA), IL-1β (#sc-52012; 1:1000; Santa Cruz Biotechnology, USA), IL-6 (#sc-32296; 1:1000; Santa Cruz Biotechnology, USA) were incubated at 4°C overnight (≥12 h), and secondary antibodies for GAPDH (#5174T; 1:5000; Cell Signalling Technology, MA, USA) and m-IgGk BP-HRP (#sc-516101; 1:5000; Santa Cruz Biotechnology, USA) were used as the internal reference antibodies. Washed with TBST 5 min × 3 times, chemiluminescence imaging was performed with ECL luminescent liquid.

**Figure 1:** The detailed flowchart of the current study.
2.9. Statistical Analyses. Using SPSS20.0 statistical analysis software, the data were expressed as means ± standard deviation (SD). The significant difference was expressed as \( p < 0.05 \). One-way ANOVA was used to compare the experimental results among groups. LSD test (meeting the requirement of homogeneity of variance) or Dunnett’s T3 (not meeting the requirement of homogeneity of variance) was used for further pairwise comparison.

3. Results

3.1. Active Components of RSRN. A total of 187 active components were identified from the TCMSP databases by the ADME thresholds (OB ≥ 30%, DL ≥ 0.18), including 8 components of *Curculigo orchioides* Gaertn (Xian Mao), 22 components of *Epimedi Folium* (Yin yang huo), 21 components of *Morindae officinalis* Radix (Ba ji tian), 2 components of *Angelicae Sinensis* Radix (Dang gui), 38 components of *Phellodendron chinense* Cortex (Huang bo), 17 components of *Anemarrhenae Rhizoma* (Zhi mu), 65 components of *Salviae Miltiorrhizae* Radix et Rhizoma (Danshen), and 14 components of *Coptidis Rhizoma* (Huang lian) (Table 1).

3.2. Analysis of “RSRN-Compound-Target” Network. We conducted target fishing for these 187 active ingredients using the TCMSP databases based on chemical similarity and obtained 225 related targets. We evaluated the relationships between the components and targets with a constructed “RSRN-compound-target” network, which had a total of 365 nodes and 4841 edges (Figure 3(a)). The inverted triangles represent the active compounds, and the circles represent their targets. The network topology was analyzed using centiscape plug-in, and the degree value of the topological network was 26.53, the betweenness value was 469.26, the closeness value was 0.0012, and the
### Table 1: Detailed information on 106 active compounds from RSRN.

| Mol ID    | Components                                      | OB%  | DL  | Herbs          |
|-----------|-------------------------------------------------|------|-----|----------------|
| MOL001506 | Supraene                                        | 33.6 | 0.42| Ba ji tian     |
| MOL002879 | Diop                                            | 43.6 | 0.39| Ba ji tian     |
| MOL002883 | Ethyl oleate (NF)                               | 32.4 | 0.19| Ba ji tian     |
| MOL00358  | Beta-sitosterol                                 | 36.9 | 0.75| Ba ji tian     |
| MOL00359  | Sitosterol                                      | 36.9 | 0.75| Ba ji tian     |
| MOL006147 | Alizarin-2-methylether                          | 32.8 | 0.21| Ba ji tian     |
| MOL009495 | 2-Hydroxy-1,5-dimethoxy-6-(methoxymethyl)-9,10-anthraquinone | 95.9 | 0.37| Ba ji tian     |
| MOL009496 | 1,5,7-Trihydroxy-6-methoxy-2-methoxymethylanthracenequinone | 80.4 | 0.38| Ba ji tian     |
| MOL009500 | 1,6-Dihydroxy-5-methoxy-2-(methoxymethyl)-9,10-anthraquinone | 105  | 0.34| Ba ji tian     |
| MOL009503 | 1-Hydroxy-3-methoxy-9,10-anthraquinone          | 104  | 0.21| Ba ji tian     |
| MOL009504 | 1-Hydroxy-6-hydroxymethylanthracenequinone      | 81.8 | 0.21| Ba ji tian     |
| MOL009513 | 2-Hydroxy-1,8-dimethoxy-7-methoxymethylanthracenequinone | 112  | 0.37| Ba ji tian     |
| MOL009519 | (2R,3S)-(-)-3',5-dihydroxy-4,7-dimethoxydihydroflavonol | 77.2 | 0.33| Ba ji tian     |
| MOL009524 | 3beta,20(R),5-alkenyl-stigmastol                | 36.9 | 0.75| Ba ji tian     |
| MOL009525 | 3beta-24S(R)-butyl-5-alkenyl-cholesterol        | 35.4 | 0.82| Ba ji tian     |
| MOL009537 | Americanin A                                    | 46.7 | 0.35| Ba ji tian     |
| MOL009541 | Asperuloside tetraacetate                       | 45.5 | 0.82| Ba ji tian     |
| MOL009551 | Isoprinsepin                                    | 49.1 | 0.77| Ba ji tian     |
| MOL009558 | 2-Hydroxyethyl 5-hydroxy-2-(2-hydroxybenzoyl)-4-(hydroxymethyl)benzoate | 62.3 | 0.26| Ba ji tian     |
| MOL009562 | Ohioensin-A                                     | 38.1 | 0.76| Ba ji tian     |
| MOL000358 | Beta-sitosterol                                 | 36.9 | 0.75| Dang gui       |
| MOL000449 | Stigmasterol                                    | 43.8 | 0.76| Dang gui       |
| MOL00454  | Berberine                                       | 36.9 | 0.78| Huang bo       |
| MOL00458  | Coptisine                                       | 30.7 | 0.86| Huang bo       |
| MOL002636 | Khadalactone A                                  | 34.2 | 0.82| Huang bo       |
| MOL013352 | Obacunone                                       | 43.3 | 0.77| Huang bo       |
| MOL002641 | Phellavin,qt                                    | 35.9 | 0.44| Huang bo       |
| MOL002643 | Delta 7-stigmasterol                            | 37.4 | 0.75| Huang bo       |
| MOL002644 | Phellopterin                                    | 40.2 | 0.28| Huang bo       |
| MOL002651 | Dehydrotanshinone II A                          | 43.8 | 0.4 | Huang bo       |
| MOL002652 | delta7-dehydrophosphoramine                     | 54.5 | 0.25| Huang bo       |
| MOL002656 | Dihydroiloticin                                 | 36.4 | 0.81| Huang bo       |
| MOL002659 | Khadanin A                                      | 31.6 | 0.7 | Huang bo       |
| MOL002660 | Niloticin                                       | 41.4 | 0.82| Huang bo       |
| MOL002662 | Rutacearpine                                    | 40.3 | 0.6 | Huang bo       |
| MOL002663 | Skimmianin                                      | 40.1 | 0.2 | Huang bo       |
| MOL002666 | Chelerythrine                                   | 34.2 | 0.78| Huang bo       |
| MOL000449 | Stigmasterol                                    | 43.8 | 0.76| Huang bo       |
| MOL002668 | Worenine                                        | 45.8 | 0.87| Huang bo       |
| MOL002670 | Cavidine                                        | 35.6 | 0.81| Huang bo       |
| MOL002671 | Candletoxin A                                   | 31.8 | 0.69| Huang bo       |
| MOL002672 | Hericenone H                                    | 39   | 0.63| Huang bo       |
| MOL002673 | Hispidone                                       | 36.2 | 0.83| Huang bo       |
| MOL000358 | Beta-sitosterol                                 | 36.9 | 0.75| Huang bo       |
| MOL000622 | Magnograndiolide                                | 63.7 | 0.19| Huang bo       |
| MOL000762 | Palmidin A                                      | 35.4 | 0.65| Huang bo       |
| MOL000785 | Palmitine                                       | 64.6 | 0.65| Huang bo       |
| MOL000787 | Fumarine                                        | 59.3 | 0.83| Huang bo       |
| MOL000790 | Isoerypalmine                                   | 35.8 | 0.59| Huang bo       |
| MOL000998 | Quercetin                                       | 46.4 | 0.28| Huang bo       |
| MOL001131 | Phellamurin,qt                                  | 56.6 | 0.39| Huang bo       |
| MOL001455 | (S)-canadine                                    | 53.8 | 0.77| Huang bo       |
| MOL001771 | Poriferast-5-en-3beta-ol                       | 36.9 | 0.75| Ba ji tian     |
| MOL001601 | 1,2,5,6-Tetrahydrotanshinone                    | 38.75 | 0.36| Dan shen      |
| MOL001659 | Poriferasterol                                  | 43.83 | 0.76| Dan shen      |
| MOL001771 | Poriferast-5-en-3beta-ol                       | 36.91 | 0.75| Dan shen      |
| MOL001942 | Isoimperatorin                                  | 45.46 | 0.23| Dan shen      |
| MOL002222 | Sugiol                                          | 36.11 | 0.28| Dan shen      |
| MOL002651 | Dehydrotanshinone II A                         | 43.76 | 0.4 | Dan shen      |
| MOL002776 | Baicalin                                        | 40.12 | 0.75| Dan shen      |
| Mol ID     | Components                                                                 | OB%  | DL | Herbs       |
|-----------|------------------------------------------------------------------------------|------|----|-------------|
| MOL000569 | Digallate                                                                    | 61.85| 0.26| Dan shen    |
| MOL000006 | Luteolin                                                                     | 36.16| 0.25| Dan shen    |
| MOL006824 | α-Amyrin                                                                     | 39.51| 0.76| Dan shen    |
| MOL007036 | 5,6-Dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one          | 33.77| 0.29| Dan shen    |
| MOL007041 | 2-Isopropyl-8-methylphenanthrene-3,4-dione                                    | 40.86| 0.23| Dan shen    |
| MOL007045 | 3α-Hydroxytanshinonea                                                        | 44.93| 0.44| Dan shen    |
| MOL007048 | (E)-3-[(2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl)acrylic acid      | 48.24| 0.31| Dan shen    |
| MOL007049 | 4-Methylenemiltirone                                                         | 34.35| 0.23| Dan shen    |
| MOL007050 | 2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofuranboxaldehyde | 62.78| 0.4 | Dan shen    |
| MOL007051 | 6-O-Syringyl-8-o-acetyl shanzhiside methyl ester                             | 46.69| 0.71| Dan shen    |
| MOL007058 | Formyltanshinone                                                             | 73.44| 0.42| Dan shen    |
| MOL007059 | 3-Beta-hydroxyangletanshinquinone                                             | 32.16| 0.41| Dan shen    |
| MOL007061 | Methyleneoxytanshinquinone                                                   | 37.07| 0.36| Dan shen    |
| MOL007063 | Przewalskin a                                                                | 37.11| 0.65| Dan shen    |
| MOL007064 | Przewalskin b                                                                | 110.32| 0.44| Dan shen    |
| MOL007068 | Przewaquinone B                                                              | 62.24| 0.41| Dan shen    |
| MOL007069 | Przewaquinone c                                                              | 55.74| 0.4  | Dan shen    |
| MOL007070 | (6S,7 R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7-naphtho[8,7-g] benzofuran-10,11-dione | 41.31| 0.45| Dan shen    |
| MOL007071 | Przewaquinone f                                                              | 40.31| 0.46| Dan shen    |
| MOL007077 | Scareol                                                                      | 43.67| 0.21| Dan shen    |
| MOL007079 | Tanshinaldehyde                                                              | 52.47| 0.45| Dan shen    |
| MOL007081 | Danshenol B                                                                  | 57.95| 0.56| Dan shen    |
| MOL007082 | Danshenol A                                                                  | 56.97| 0.52| Dan shen    |
| MOL007085 | Salvilene                                                                    | 30.38| 0.38| Dan shen    |
| MOL007088 | Cryptotanshinone                                                             | 52.34| 0.4  | Dan shen    |
| MOL007093 | Dan-shexinkum d                                                               | 38.88| 0.55| Dan shen    |
| MOL007094 | Danshenspiroketallactone                                                    | 50.43| 0.31| Dan shen    |
| MOL007098 | Deoxyneocryptotanshinone                                                     | 49.4 | 0.29| Dan shen    |
| MOL007100 | Dihydrotanshinlactone                                                        | 38.68| 0.32| Dan shen    |
| MOL007101 | Dihydrotanshinone I                                                          | 45.04| 0.36| Dan shen    |
| MOL007105 | Epidanshenspiroketallactone                                                  | 68.27| 0.31| Dan shen    |
| MOL002331 | N-Methylflindersine                                                           | 32.4 | 0.18| Huang bo    |
| MOL002894 | Berberrubine                                                                  | 35.7 | 0.73| Huang bo    |
| MOL005438 | Campesterol                                                                   | 37.6 | 0.71| Huang bo    |
| MOL006392 | Dihydroniloticin                                                             | 36.4 | 0.82| Huang bo    |
| MOL006401 | Melianone                                                                     | 40.5 | 0.78| Huang bo    |
| MOL006413 | Phellochin                                                                   | 35.4 | 0.82| Huang bo    |
| MOL006422 | Thalifendine                                                                  | 44.4 | 0.73| Huang bo    |
| MOL001607 | ZINC03982454                                                                 | 36.9 | 0.76| Xian Mao    |
| MOL003578 | Cycloartenol                                                                  | 38.7 | 0.78| Xian Mao    |
| MOL003358 | Beta-sitosteroster                                                            | 36.9 | 0.75| Xian Mao    |
| MOL004114 | 3,2′,4′,6′-tetrahydroxy-4,3′-dimethoxy chalcone                               | 52.7 | 0.28| Xian Mao    |
| MOL004125 | Curculigoside B_qt                                                            | 83.4 | 0.19| Xian Mao    |
| MOL004146 | Curcubioside C                                                                | 39.3 | 0.19| Xian Mao    |
| MOL000449 | Stigmatic                                                                    | 43.8 | 0.76| Xian Mao    |
| MOL001510 | 24-Epicampesterol                                                             | 37.6 | 0.71| Xian Mao    |
| MOL001645 | Linoleyl acetate                                                              | 42.1 | 0.2 | Yin yang huo|
| MOL001771 | Poriferast-5-en-3beta-ol                                                      | 36.9 | 0.75| Yin yang huo|
| MOL001792 | DFV                                                                          | 32.8 | 0.18| Yin yang huo|
| MOL003444 | Chryseriol                                                                    | 35.9 | 0.27| Yin yang huo|
| MOL003542 | 8-Isopentenyl-kaempferol                                                      | 38  | 0.39| Yin yang huo|
| MOL003559 | Sitosterol                                                                    | 36.9 | 0.75| Yin yang huo|
| Mol ID    | Components                                      | OB% | DL | Herbs       |
|----------|------------------------------------------------|-----|----|-------------|
| MOL000422 | Kaempferol                                      | 41.9| 0.24| Yin yang huo|
| MOL004367 | Olivil                                          | 62.2| 0.41| Yin yang huo|
| MOL004373 | Anhydroicaritin                                 | 45.4| 0.44| Yin yang huo|
| MOL004380 | C-Homoerythrinan,1,6-didehydro-3,15,16-trimethoxy-, (3. Beta)- | 39.1| 0.49| Yin yang huo|
| MOL004382 | Yin yang huo A                                 | 57  | 0.77| Yin yang huo|
| MOL004384 | Yin yang huo C                                 | 45.7| 0.5 | Yin yang huo|
| MOL004386 | Yin yang huo E                                 | 51.6| 0.55| Yin yang huo|
| MOL004388 | 6-Hydroxy-11,12-dimethoxy-2,2-dimethyl-1,8-dioxo-2,3,4,8-tetrahydro-1h-isochromeno[3,4-h] isoquinolin-2-ium | 60.6| 0.66| Yin yang huo|
| MOL004391 | 8-(3-Methylbut-2-enyl)-2-phenyl-chromone         | 48.5| 0.25| Yin yang huo|
| MOL004394 | Anhydroicaritin-3-O-alpha-L-rhamnoside          | 41.6| 0.61| Yin yang huo|
| MOL004396 | 1,2-bis(4-hydroxy-3-methoxyphenyl) propan-1,3-diol | 52.3| 0.22| Yin yang huo|
| MOL004425 | Icariin                                        | 41.6| 0.61| Yin yang huo|
| MOL004427 | Icariside A7                                   | 31.9| 0.86| Yin yang huo|
| MOL000006 | Luteolin                                       | 36.2| 0.25| Yin yang huo|
| MOL000622 | Magnograndiolide                               | 63.7| 0.19| Yin yang huo|
| MOL000098 | Quercetin                                       | 46.4| 0.28| Yin yang huo|
| MOL001677 | Asperglaucide                                   | 58  | 0.52| Zhi mu     |
| MOL001944 | Marmesin                                        | 50.3| 0.18| Zhi mu     |
| MOL003773 | Mangiferolic acid                               | 36.2| 0.84| Zhi mu     |
| MOL000422 | Kaempferol                                      | 41.9| 0.24| Zhi mu     |
| MOL004373 | Anhydroicaritin                                 | 45.4| 0.44| Zhi mu     |
| MOL004489 | Anemarsaponin F_qt                             | 60.1| 0.79| Zhi mu     |
| MOL004492 | Chrysanthenmaxanthin                           | 38.7| 0.58| Zhi mu     |
| MOL004497 | Hippeastrine                                    | 51.7| 0.62| Zhi mu     |
| MOL004514 | Timosaponin B III_qt                           | 35.3| 0.87| Zhi mu     |
| MOL000449 | Stigmasterol                                    | 43.8| 0.76| Zhi mu     |
| MOL004528 | Icarin I                                        | 41.6| 0.61| Zhi mu     |
| MOL004540 | Anemarsaponin C_qt                             | 35.5| 0.87| Zhi mu     |
| MOL004542 | Anemarsaponin E_qt                             | 30.7| 0.86| Zhi mu     |
| MOL000483 | (Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl) ethyl] acrylamide | 118 | 0.26| Zhi mu     |
| MOL000546 | Diosgenin                                       | 80.9| 0.81| Zhi mu     |
| MOL000631 | Coumaroyltetramine                             | 113 | 0.2 | Zhi mu     |
| MOL007107 | CO9092                                         | 36.07| 0.25| Dan shen  |
| MOL007108 | Isocryptotanshi-none                           | 54.98| 0.39| Dan shen  |
| MOL007111 | Isotashinhone II                              | 49.92| 0.4  | Dan shen  |
| MOL007115 | Manool                                         | 45.04| 0.2  | Dan shen  |
| MOL007118 | Microstegiol                                   | 39.61| 0.28| Dan shen  |
| MOL007119 | Miltionone I                                  | 49.68| 0.32| Dan shen  |
| MOL007120 | Miltionone II                                 | 71.03| 0.44| Dan shen  |
| MOL007121 | Miltipolone                                    | 36.56| 0.37| Dan shen  |
| MOL007122 | Miltirone                                     | 38.76| 0.25| Dan shen  |
| MOL007123 | Miltirone II                                   | 44.95| 0.24| Dan shen  |
| MOL007124 | Neocryptotanshinone ii                       | 39.46| 0.23| Dan shen  |
| MOL007125 | Neocryptotanshinone                           | 52.49| 0.32| Dan shen  |
The top 10 compounds and top 6 key target of RSRN are shown in Table 2. The top six targets were PTGS2 (degree = 231), PTGS1 (degree = 148), SCN5A (degree = 137), HSP90AA1 (degree = 131), NCOA2 (degree = 130), and ADRB2 (degree = 127) (Figure 3(b) and 3(c)).

3.3. Disease Targets Acquisition and Analysis. There were 1373 disease targets related to hyperlipidemia, 4481 targets related to atherosclerosis, and 9553 targets related to "hyperlipidemia," which were selected from GeneCards, TTD, DrugBank, and OMIM database. The above disease targets were analyzed by Venn map (Figure 4(a)). The data set GSE57691 was selected from the GEO database for screening differentially expressed genes. Compared with control samples, a total of 269 genes were significantly differentially expressed in hyperlipidemia samples, 47 were upregulated, and 221 were downregulated. The differentially expressed genes are shown in the cluster diagram (Figure 4(b)) and volcano map (Figure 4(c)). A total of 974 disease targets were obtained by merging above disease targets in the end.

3.4. Construction of Drug-Disease Target Network. After the construction of the Venn diagram, 106 targets between the 974 disease targets and 225 related targets of RSRN were selected as the potential targets in the treatment of hyperlipidemia-related diseases (Figure 5(a)). The protein interaction relationship was obtained by using BisoGenet plug-in of Cytoscape software (Figure 5(b)). Using the CytoNCA plug-in of Cytoscape software, the core disease-drug targets was analyzed and confirmed. The selection criteria were set as follows: DC value > 61 (Figure 5(c)) (yellow part in the figure was qualified), and BC value > 600 (Figure 5(d)). The red rectangle in the topological network represents the last selected target genes, including AKT1, AR, NF-κB, CASP3, mTOR, ERBB2, CHUK, CAV1, MAPK8, MAPK14, HIF1A, PPARG, RELA, NR3C1, ESR2, FOS, CDK4, GSK3β, HSPB1, MYC, MDM2, EGFR, HSP90AA1, HSPA5, NOS2, ADRB2, VCAM1, APP, ESR1, XIAP, CASP8, Bax, ICAM1, SOD1.

3.5. Functional Enrichment Analysis. The 106 potential targets were then subjected to GO and KEGG analysis to explore the links between the functional units, their potential significance in the biological systems network. The GO terms were determined in the following categories (Figures 6(a) and 6(b): 1900 biological processes (BP), 43 cellular components (CC), and 140 molecular functions (MF) branches. In the category BP, the genes were associated with response to metal ion (GO:0006038), response to nutrient levels (GO:0031667), response to lipopolysaccharide (GO:0032496), and response to molecular of bacterial origin (GO:0002237). In the category CC, the genes were associated with cell components such as membrane raft (GO:0045121), membrane micro domain (GO:0098857), membrane region (GO:0098589), and transcription regulator complex (GO:0006355).

### Table 1: Continued.

| Mol ID    | Components                                                                 | OB%   | DL   | Herbs  |
|-----------|-----------------------------------------------------------------------------|-------|------|--------|
| MOL007127 | 1-Methyl-8,9-dihydro-7h-naphtho[5,6-g] benzofuran-6,10,11-trione              | 34.72 | 0.37 | Dan shen |
| MOL007130 | Prolithospermic acid                                                         | 64.37 | 0.31 | Dan shen |
| MOL007132 | (2R)-3-(3,4-dihydroxyphenyl)-2-[((Z)-3-(3,4-dihydroxyphenyl) acryloyl] oxy-propionic acid | 109.38 | 0.35 | Dan shen |
| MOL007140 | (Z)-3-[2-((E)-2-(3,4-dihydroxyphenyl) vinyl]-3,4-dihydroxy-phenyl] acrylic acid | 88.54 | 0.26 | Dan shen |
| MOL007141 | Salvianolic acid g                                                           | 45.56 | 0.61 | Dan shen |
| MOL007142 | Salvianolic acid j                                                           | 43.38 | 0.72 | Dan shen |
| MOL007143 | Salvileneone I                                                               | 32.43 | 0.23 | Dan shen |
| MOL007145 | Salviolone                                                                   | 31.72 | 0.24 | Dan shen |
| MOL007149 | NSC 122421                                                                   | 34.49 | 0.28 | Dan shen |
| MOL007150 | (6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7h-naphtho[8,7-g] benzofuran-10,11-quinone | 75.39 | 0.46 | Dan shen |
| MOL007151 | Tanshindiol B                                                                | 42.67 | 0.45 | Dan shen |
| MOL007152 | Przewaquinone E                                                              | 42.85 | 0.45 | Dan shen |
| MOL007154 | Tanshinone iia                                                               | 49.89 | 0.4   | Dan shen |
| MOL007155 | (6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7h-naphtho[8,7-g] benzofuran-10,11-dione | 65.26 | 0.45 | Dan shen |
| MOL007156 | Tanshinone VI                                                                | 45.64 | 0.3   | Dan shen |
| MOL002897 | Epiberberine                                                                 | 43.09 | 0.78 | Huang lian |
| MOL002903 | (R)-canadine                                                                 | 55.37 | 0.77 | Huang lian |
| MOL002904 | Berlambine                                                                   | 36.68 | 0.82 | Huang lian |
| MOL002907 | Corchoroside A_qt                                                            | 104.95 | 0.78 | Huang lian |
| MOL000622 | Magnograncondiolide                                                          | 63.71 | 0.19 | Huang lian |
| MOL000762 | Palmidin A                                                                   | 35.36 | 0.65 | Huang lian |
| MOL000785 | Palmatine                                                                    | 64.6  | 0.65 | Huang lian |
| MOL000908 | Quercetin                                                                    | 46.43 | 0.28 | Huang lian |
| MOL001458 | Coptisine                                                                    | 30.67 | 0.86 | Huang lian |
| MOL002668 | Worenine                                                                      | 45.83 | 0.87 | Huang lian |
| MOL008647 | Moupinamide                                                                   | 86.71 | 0.26 | Huang lian |
In the category MF, the genes were associated with DNA binding transcription factor binding (GO:0140297) and RNA polymerase II specific DNA binding transcription factor binding (GO:0061629). The KEGG enrichment result indicated that the genes were associated with AGE-RAGE signaling pathway (hsa04933), fluid shear stress and atherosclerosis (hsa05162), PI3K-Akt signaling pathway (hsa04151), TNF signaling pathway (hsa04668), and NF-kappa B signaling pathway (hsa04064) (Figures 6(c) and 6(d)). The KEGG network included 83 nodes and 360 edges (Figure 7(a)). The top six target genes were AKT1 (protein-serine-threonine kinase 1) (degree = 17), RELA (nuclear factor kappa B p65, NF-κB p65) (degree = 16), CHUK (conserved helix-loop-helix ubiquitous kinase, also known as IκB kinase α, IKKα, or IKK1) (degree = 14), CCND1 (Cyclin D1) (degree = 13), MAPK8 (mitogen-activated protein kinase 8) (degree = 12), and MAPK14 (mitogen-activated protein kinase 14) (degree = 11) (Figure 7(b)). The

| Compound                     | Type | Betweenness | Closeness | Degree | Eigenvector |
|------------------------------|------|-------------|-----------|--------|-------------|
| Quercetin                    | Mol  | 6956.367    | 0.001616  | 426    | 0.172085    |
| Kaempferol                   | Mol  | 1074.972    | 0.001422  | 114    | 0.093268    |
| Stigmasterol                 | Mol  | 234.9962    | 0.001351  | 112    | 0.071736    |
| Luteolin                     | Mol  | 966.566     | 0.001414  | 110    | 0.077567    |
| Beta-sitosterol              | Mol  | 188.0214    | 0.001361  | 88     | 0.076787    |
| Anhydrocaritin               | Mol  | 173.2552    | 0.001372  | 66     | 0.083024    |
| Danshinone II A              | Mol  | 1130.871    | 0.00122   | 41     | 0.073208    |
| Dehydrotanshinone II A       | Mol  | 26.81371    | 0.001348  | 40     | 0.06437     |
| Palmatine                    | Mol  | 16.99011    | 0.001297  | 34     | 0.054885    |
| C-homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-β, (3. Beta) - | Mol  | 292.7947    | 0.001333  | 32     | 0.070996    |
| PTGS2                        | Gene | 6853.524    | 0.001597  | 231    | 0.158477    |
| PTGS1                        | Gene | 1835.112    | 0.001504  | 148    | 0.126258    |
| SCN5A                        | Gene | 1382.629    | 0.001497  | 137    | 0.127246    |
| HSP90AA1                     | Gene | 2411.688    | 0.001497  | 131    | 0.107942    |
| NCOA2                        | Gene | 2395.628    | 0.001481  | 130    | 0.098189    |
| ADRB2                        | Gene | 1468.898    | 0.001495  | 127    | 0.12509     |
PI3K-Akt signaling pathway and NF-kappa B signaling pathway were performed by R software (Figures 7(c) and 7(d)), and the red mark represents the potential target of RSRN intervention.

3.6. RSRN Downregulated the Expression of NF-κBp65 Protein in Hypothalamus of Ovariectomized Rats. The immunohistochemistry results showed that the positive expression of NF-κBp65 cells included glial cells, which were located in cytoplasm or nucleus (Figure 8(a)–8(e)). Compared with the SHAM group, the expression of NF-κBp65 protein in hypothalamus in OVX group was significantly increased ($p < 0.05$), with a large number of positive expression cells and dark brown color. Compared with the OVX group, the expression of NF-κBp65 protein in hypothalamus in RSRN group and EV group was significantly decreased ($p < 0.05$).

3.7. RSRN Regulated the Expression of NF-κBp65, IκBα, TNFα, IL-1β, and IL-6 in Brain of Ovariectomized Rats. Western blotting results showed that the expression levels of NF-κBp65, TNFα, IL-1β, and IL-6 in RSRN group were significantly decreased ($p < 0.05$), while the expression of IκBα protein was significantly increased ($p < 0.05$) (Figure 9(a)–9(c); Figure 10(a)–10(c)).

4. Discussion

The method of reducing the south and reinforcing the north (RSRN) can nourish kidney essence and purging the heart. It is also called the method of purging fire and replenishing water. The method of RSRN in this study was used to coordinate yin and yang and to prevent postmenopausal-related diseases. There were 187 active components in RSRN, of which quercetin, kaempferol, Stigmasterol, luteolin, beta-sitosterol, and Anhydroicaritin were the main active components. Kaempferol and quercetin may have hypoglycemic, lipid-lowering, anti-inflammatory, antioxidant, and anticancer effects [17]. It was reported that Kaempferol can increase lipid metabolism by increasing PPARα level, decreasing SREBPs level, and promoting expression of ACO and CYP4A1, so as to reduce visceral fat accumulation and improve hyperlipidemia in obese rats fed with high-fat diet [18]. Quercetin can improve cholesterol reverse transport by upregulating the expression of ABCA1 and ABCG1 protein and enhancing the cholesterol acceptance of HDL and LDL.
ApoA1 by reducing oxidation so as to reduce lipid accumulation [19]. Research [20] showed that luteolin could reduce the activation of PI3K/Akt induced by EGF and reduce the phosphorylation of EGFR, Akt, p38, and extracellular signal regulated kinase (ERK). Studies [21] found that pretreatment with luteolin could reduce the production of proinflammatory cytokines such as TNF-α, IL-6, and inflammatory mediator nitric oxide (NO) which produced by lipopolysaccharide (LPS)-stimulated MH-S cells of mouse alveolar macrophages. Studies have illustrated that β-sitosterol exerts cholesterol-lowering, antioxidant, and anti-inflammatory effects [22, 23]. The above studies showed that the main active ingredients of RSRN had anti-inflammatory, antioxidant stress, hypoglycemic and lipid-lowering effects, and cardiovascular system protection.

A total of 106 drug-disease core targets were obtained, and the genes were used to constructed the core target gene topology network and the KEGG network. The top six target genes from the KEGG network were merged with the candidate genes from the core target gene topology network; we obtained the key target genes including AKT1, RELA, CHUK, MAPK8, MAPK14, and CCND1. The key targets are mainly involved in NF-κB/MAPK signaling pathway, PI3K-Akt signaling pathway, atherosclerosis, and inflammation-related signaling pathways. Akt is the central link of PI3K/Akt signaling pathway, and it plays an important role in regulating cell survival, protein synthesis, angiogenesis, and insulin-dependent metabolic cell response [24]. Akt can inhibit apoptosis, phosphorylate caspase-9 precursor [25, 26]. Nuclear factor κB (NF-κB) plays an important role in the regulation of gene transcription related to inflammation, cell proliferation, differentiation and apoptosis, immune response, and tumor formation; the human NF-κB family consists of five members: P50/P105, p52/P100, p65/RELA, RELB, and c-Rel, which are encoded by NF-κB1, NF-κB2, RELA, RELB, and REL genes [27]. CHUK (also known as IKK α, IKK α) is the upstream component of signal transduction pathway that directly enters the nucleus to regulate gene expression, and it is also a component of activating cytokine protein complex, studies have shown that gene mutation of CHUK is associated with hypertension and lipid abnormality [28–30]. These studies indicated that the key targets have the functions of regulating lipid metabolism, glucose metabolism, and immune regulation, which were of great significance for the prevention and treatment of hyperlipidemia, and also had certain effects on the complications of atherosclerosis and Alzheimer’s disease.

The core targets of RSRN-active ingredients in the treatment of hyperlipidemia-related diseases may involve in the response to lipopolysaccharide, oxidative stress, and DNA binding transcription factor binding. Oxidative stress plays an important role in the pathogenesis of atherosclerosis (AS), hypertension, metabolic syndrome, hypercholesterolemia, Alzheimer’s disease, aging, and cancer [31]. Lipopolysaccharide (LPS) activates

![Figure 5: The drug-disease target network. (a) The Venn diagram for drug and disease targets. The overlap targets mean the potential therapeutic gene for RSRN when treating hyperlipidemia-related disease; (b) there were 6664 nodes and 146555 edges in the network; (c) the first screening threshold was DC > 61, which resulted in 1350 nodes and 58313 edges; (d) the second screening threshold was BC > 600, and 609 nodes and 25745 edges remained.](image-url)
downstream target genes by activating TLR4/NF-κB signaling pathways, thus to release inflammatory factors such as TNF-α, IL-8, IL-1, and IL-6 [32]. Endotoxin is an inflammatory reaction promoter of LPS in the outer membrane of Gram-negative bacteria; it is the main ligand of Toll like receptor; and it has been confirmed that endotoxin plays an important role in the process and progress of AS [33, 34].

The main pathways of RSRN may be involved in AGE-RAGE signaling pathway, cell fluid shear stress and atherosclerosis, and PI3K-Akt signaling pathway. Advanced glycosylated compounds (AGEs) are complex compounds produced by nonenzymatic glycosylation and oxidation of proteins, lipids, and nucleic acids; they can activate AGE-RAGE signaling pathway, MAPK signaling pathway, and NF-κB signaling pathway, leading to the expression of proinflammatory cytokines such as IL-1, IL-6, and TNF-α, and the release of VCAM1, VEGF, and RAGE, so as to promote the development of atherosclerosis [35]. Cell fluid shear stress and atherosclerotic pathway play an important role in the development of dyslipidemia to atherosclerosis; it has been reported that the wall concentration of lipid in the slow flow area was higher than that in the high-speed laminar flow area, and the action time of lipid and arterial wall was prolonged, which was easy to cause atherosclerosis, and it also can promote oxidative stress, increase the production of ox LDL, upregulate the expression of NF-κB, thus to promote inflammatory response [36, 37]. PI3K/Akt signaling pathway plays an important role in lipid metabolism and inflammation regulation; inhibition of PI3K/Akt signaling pathway can significantly reduce serum-free fatty acids, cholesterol, and triglyceride [38, 39], and it also inhibited the secretion of proinflammatory mediators such as TNF-α and IL-1 β [40]. It can be speculated that RSRN may regulate dyslipidemia and prevent or delay the occurrence and development of AS through regulation of endocrine, metabolic, and inflammatory pathways.

Studies have found that hyperlipidemia is closely related to hypothalamic inflammatory response. Hypothalamic inflammation leads to the occurrence of obesity-based metabolic diseases. A short-term high-fat diet can increase the expression of biomarkers and promote inflammatory response in the basal hypothalamus to form a transient inflammation. Under the condition of long-term high-fat diet, hypothalamic glial hyperplasia, and nerve injury can promote the occurrence of hypothalamic inflammation [41]. In this experimental study, the results showed that the expression of NF-κB p65, TNF-α, IL-1β, IL-6 in hypothalamic nucleus of OVX group was

**Figure 6**: Functional enrichment analysis. (a, b) GO functional enrichment analysis; (c, d) KEGG functional enrichment analysis.
significantly increased, which indicated that NF-κB signal pathway and inflammatory cytokines were activated under the stimulation of intracellular and extracellular signals; after treatment with RSRN, the expression of activated NF-κB (p65) in nucleus was significantly reduced, and the expression of TNF-α, IL-1β, and IL-6 was also significantly reduced in RSRN group; the results showed that RSRN could inhibit the activity of NF-κB and reduce the release of inflammatory cytokines. In the future, it is necessary to further explore the relationship between target protein and the upstream and downstream molecules of the signaling pathway and the specific regulatory mechanisms and confirm the curative effect through clinical trials.
Figure 8: The expression of NF-κBp65 in hypothalamus. (a–d) The expressions of NF-kBp65 in hypothalamus tissue were analyzed by immunohistochemistry (×200). (e) Values are presented as the mean ± standard deviation (SD), n = 3 per group. *p < 0.05, compared with the SHAM group; #p < 0.05, compared with the OVX group.

(a) SHAM (100×) SHAM (200×)
(b) OVX (100×) OVX (200×)
(c) RSRN (100×) RSRN (200×)
(d) EV (100×) EV (200×)
(e) NF-κBp65 (%)

Figure 9: Continued.

(a) IκBα
(b) NF-κBp65
(c) GAPDH

| hypothalamus | hippocampus |
|--------------|-------------|
| SHAM  | OVX | RSRN | EV | SHAM | OVX | RSRN | EV |

Figure 9: Continued.
Figure 9: Protein expression of IkBα and NF-κBp65 by western blot: (a) gene levels of NF-κBp65, IkBα in hypothalamus and hippocampus by western blot; (b) semi-quantitative analysis of NF-κBp65, IkBα proteins expression compared with GAPDH. Values are presented as the means± standard deviation (SD), n = 3 per group. ★ p < 0.05, compared with the SHAM group; # p < 0.05, compared with the OVX group.

Figure 10: Continued.
5. Conclusion

In this study, a total of 187 potential active components and 106 related core targets were obtained and identified overall. Then after the Metascape enrichment analysis, RSRN may regulate AKT1, NF-κBp65, IKK α, TNF-α, IL-1β, IL-6 through TNF signaling pathway, PI3K-Akt signaling pathway, and NF-kappa B signaling pathway, so as to regulate lipid metabolism, inflammatory response, and prevent or delay the development of atherosclerotic diseases. This study suggests that RSRN may be used in the treatment of hyperlipidemia and related diseases. Due to the limitation of database data and corresponding analysis algorithms, the results may be biased, and further in vitro and in vivo studies are needed to verify the results.

Abbreviations

ADME: Absorption, distribution, metabolism, and excretion

Data Availability

All the data generated or analyzed during this study are included within the paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.
Authors’ Contributions

Hongjin Wu conceived and designed the experiment. Weiwei Dai analyzed the data and edited the manuscript. Jie Zhang, Libo Wang, and Chenglong Wang were responsible for figure drawing and table design and the manuscript revising. All the authors have read and approved the final manuscript.

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