Utilizing Network Pharmacology to Explore the Possible Mechanism of CoptidisRhizoma in Kawasaki Disease

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Research

Keywords: Kawasaki disease, Network pharmacology, CoptidisRhizoma

Posted Date: October 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-90022/v1

Abstract

Background: Kawasaki disease (KD) isan acute self-limiting systemic vasculitis.In this study, a randomized controlled trial regarding berberine (main component of CoptidisRhizoma) function in treating KD was carried out and possible pharmacological mechanisms of CoptidisRhizoma (CR) on Kawasaki disease therapy were investigated using an integrated network pharmacology approach.

Results: The BBR group was able to reduce the values of CRP, NLR and PLR significantly. Also, the effect of BBR improved the resistance rate of intravenous injection of gamma globulin significantly. In total, 9 compounds and 369 relative drug targets were collected from TCMSP, SWISS, SEA and STITCH database and 624 KD target genes were collected in DisGeNET, DrugBank and GeneCards database. The network analysis revealed that 41 targets might be the therapeutic targets of CR on KD, among which ATK1, RELA, SRC, CASP3 and MTOR ranked in top 5. Gene ontology enrichment analysis revealed that the reaction to bacteria-derived molecules and to lipopolysaccharide and the apoptosis process were the key biological procedures for CR treating KD. The KEGG pathway enrichment analysis pointed out that the four signaling pathways closely related to CR treating KD including age-rage signaling pathway, fluid shear stress and atherosclerosis, TNF signaling pathway and Toll-like receptor signaling pathway in diabetic complications.

Conclusion: We concluded that the introduction of routine treatment combined with BBR in treating KD has advantages than routine treatment and can be considered as a preferred approach in KD. Network pharmacology showed that CR exerted the effect of prevention KD by regulating multi-targets and multi-components.

Background

Kawasaki disease (KD) is a systemic inflammatory vasculitis predominantly affecting children younger than 5 years of age [1] and is now the most common acquired heart disease among children in North America, Europe and Japan [2]. KD can cause permanent vascular complications, especially coronary artery aneurysms (CAA) that may result in myocardial infarction [3, 4] and may also be associated with serious cardiovascular sequelae in adulthood [5]. The standard treatment for Kawasaki disease includes intravenous gamma globulin and high-dose aspirin. However, up to 15–20% of patients with Kawasaki disease do not respond to IVIG treatment and have an increased rate of CAA [6]. Yet, the underlying mechanisms of KD remain unclear and it may be related to unbalanced autoimmunity and genetic susceptibility[7]. It is important to systematically elucidate the mechanism of the disease and search for more effective agents against it.

CoptidisRhizoma(CR), called Huang Lian (HL) in Chinese, belongs to perennial herbs of Ranunculaceae. The Isoquinoline Alkaloids in CoptisChinensis, namely Berberine (BBR), CoptisChinensis and Rhizopine, are the main effective components of CR. Data have proved that CR has anti-inflammatory [8], antibacterial [9], antitumor [10], antidiabetics [11] and pharmacological effects on cardiovascular system,

mainly related to the effects of BBR. A previous study in the same field has reported that BBR protected endothelial progenitor cells from injury induced by tumor necrosis factor α via the Phosphatidyl Inositol 3-kinase (PI3K)/serine/threonine protein kinase B /endothelial nitric oxide synthase signaling pathway [12]. In addition, another examination confirmed that BBR protects KD-induced human coronary artery endothelial cells dysfunction by inhibiting oxidative and endoplasmic reticulum stress [13].

Network pharmacology is a part of bioinformatics based on the concept of "disease - gene - target - drug" [14], which is a new and effective means to study the mechanisms of drug therapy[15]. Using network pharmacology, the relationship between drugs and diseases is analyzed from molecular level, multi-target and multi-pathway, potentially providing useful information in the search for drug treatment.

In order to investigate the clinical effects of BBR on patients with KD and its pharmacological mechanisms, a randomized and case control study was performed in Shenzhen Children's Hospital and a comprehensive network pharmacology approach was established to probe the appliances of CR on KD by network pharmacology analysis.

Results

Clinical Research outcomes

One of the first things to be observed in this phase is that no statistically significant differences were found in baseline data and hematologic indicators between the two groups. The BBR group was able to reduce the values of c-reactive protein (CRP), neutrophils/lymphocytes (NLR) and platelets/lymphocytes (PLR)(Fig.1-3), and there was no statistical divergence in the number of days with fever, WBC, PLT, AST, ALT and other blood indexes between the samples. Also, the resistance rate of IVIG decreased after in BBR group.

Active compounds in CR

Overall, 9 target compounds were collected from TCMSP and 369 target genes in the whole formula in total were retrieved from TCMSP、SWISS、SEA、STITCH database (Fig.4). The values of OB, DL and BBB were used to screen potential active compounds and the detailed properties of the compounds were shown in the supplemental file 1.

Target screening of KD

A total of 624 significant genes were gathered from the DisGeNET、DrugBank、GeneCards databases after deleting repetitions (Fig.5). Kawasaki disease was used as a key word for retrieval, screening and sorting out related targets and the Venn diagram was applied to show the intersection of disease targets and component targets. Finally, 41 therapeutic targets of CR for KD could be obtained (Fig.6).

Compound–compound target network analysis

To further determine the target of CR on KD, a compound-target network was built with an online tool Cytoscape3.6, representing a compound-target genes network (Figure.7). Meanwhile, the results were shown in the supplemental file 1.

GO and KEGG pathway enrichment analysis

To investigate the biological functions of the direct targets of CR for KD, the gene GO biological process (BP) was performed by the Cytoscape3.6.0 plugin ClueGO. GO enrichment analysis showed that these biological processes were mainly involved in the reaction to bacteria-derived molecules apoptosis which may be the key biological processes for CR prevention and control of KD (Fig.8). The functional annotation tool of DAVID was used for KEGG pathway enrichment analysis. Ultimately, 10 signaling pathways were screened, including Age-rage signaling pathways, fluid shear stress and atherosclerosis, TNF signaling pathways, and tol1-like receptor signaling pathways in diabetic complications. All of them are displayed in Fig.9. Meanwhile, Cytoscape3.6.0 software was used to demonstrate the 41 proteins enrichment of key pathways (Fig.10).

Compound–common target between compound and KD network analysis

For this part of the study, the String online server was the main tool that helped to build the PPI network, containing 41 nodes in total, as shown in Fig.11. Finally, the significant target genes were screened according the degree and ranked in the top 10 (Table1).

Discussion

Kawasaki disease is a systemic inflammatory syndrome of small and medium vessels. In recent years, more and more evidences have shown that the pathogenesis of this condition is related to infectious factors, susceptibility genes and autoimmune activation. Traditional Chinese medicine is suitable for treating diseases with complex mechanisms and, because of its multiple curative effect and small side effects. The effects of BBR on KD in clinical practice were tested based on some previous findings regarding this topic [13]. The present study can be considered the first clinical trial to evaluate the effect of BBR in patients with KD. In this study, network pharmacology method was used to analyze the possible molecular mechanisms of CR in the treatment of KD.

In the clinical trial stage,C-reactive protein (CRP) and peripheral blood cell parameters were also used as markers of systemic inflammation. Among them, CRP plays a role in promoting phagocytosis and immune regulation. Data have shown that during the course of KD, the increase of CRP level is related to coronary artery dilatation and is a high risk factor of complication with coronary artery injury for KD[16]. NLRand PLR are markers of the balance between inflammatory response and immune regulation and are associated with cardiovascular adverse events. Studies have shown that NLR is directly proportional to the intensity of inflammatory response[17]. At the same time, Turkmen et al.indicated that PLR might be more effective than NLR in predicting the severity of systemic inflammatory response[18]. The present study indicated that the BBR treatment accelerate the reduction of CRP, NLR and PLR, which means that

BBR can alleviate the inflammatory response in patients with KD. The rate of IVIG-resistance in BBR treatment group was significantly lower than that of control group, indicating that BBR increases the therapeutic efficiency of routine therapy in KD.

In order to investigate the mechanisms of BBR on KD, network pharmacology was used. 9 target compounds of CR were collected from TCMSP, 369 target genes of CR were collected from TCMSP, SWISS, SEA and STITCH database and a total of 624 target genes of CR were obtained by searching related databases together with 41 target genes obtained by the intersection of CR and KD. By further screening this sample of genes, AKT1、CASP3、TP53、MAPK1/3/8/14、PTGS2、SRC、RELA、MTOR、NOS2/3、ICAM1 were found and set as target genes as well.

AKT1, a serine/threonine protein kinase, is widely expressed in various tissues. It is known that activated AKT plays a regulatory role in cell cycle, apoptosis and proliferation by activating downstream factors. Also, the specific activation of AKT1 in vascular endothelial cells can alleviate the injury after carotid artery ligation by increasing the expression of nitric oxide and protecting the function of endovascular cortex [19]. By initializing AKT1 in vascular smooth muscle cells it is possible to effectively inhibit the apoptosis and negative remodeling of vascular smooth muscle cells after carotid artery ligation, highlighting the protective role of AKT1 in vascular remodeling [20]. In addition, study have confirmed that p21 phosphorylated by AKT1 in endothelial cells may promote angiogenesis and metastasis, suggesting that p21 phosphorylation may play an important role in KD coronary artery abnormalities [21].

MAPK, an intracellular serine/threonine protein kinase, is an important signaling system for cell-mediated extracellular signals to intracellular responses, and plays a key role in cell proliferation, apoptosis, inflammation, immunity and angiogenesis [22]. Studies have found that inhibiting MAPK signaling pathway activation can reduce the occurrence of inflammatory response [23]. So, this research postulated that MAPK might play an important regulatory role in the occurrence and development of vasculitis in Kawasaki disease.

SRC encodes tyrosine protein kinase also have to be mentioned, since it is a member of the non-receptor protein tyrosine kinase family. This protein is constantly associated with multiple signaling pathways in cells and its related genes are involved in important biological processes such as growth, differentiation, adhesion and transcription. Studies have confirmed that SRC-1 gene is related to the susceptibility to coronary artery aneurysm of KD complications [24], indicating that SRC gene is linked to the regulatory mechanism of Kawasaki disease.

Additionally, CASP3 is a key apoptotic protease in the final pathway of apoptotic cell death, mediating exogenous and endogenous cell death signaling pathways. CASP3 leads to transcriptional activation of inflammatory genes by activating the NF-κB pathway in the mechanism of KD. As genetic variation of genes may also cause damage and remodeling of vascular structures, TP53 is considered to be an important tumor suppressor gene that can affect cell cycle, DNA repair, apoptosis, signal transduction, transcription and autophagy, as well as regulate the growth, differentiation and senescence of cells. PTGS2 contains 10 exons and 9 introns, encoding cyclooxygenase 2(Cox-2). Cox-2, in this case, is

expressed in vascular smooth muscle, monocytes and fibroblasts, and is a cardinal inflammatory mediator in the process of atherosclerosis [25]. In addition, overexpression of COX-2 may cause inflammation of the vascular wall, plaque instability and intimal hyperplasia [26]. It is believed that the occurrence of this vascular wall inflammation is closely related to the mechanism of KD, and this hypothesis is consistent with the results of the network pharmacology processes here conducted.

RELA is a member of the NF-κB family. NF-κB plays a key role in inflammatory and immune responses in cells. Studies have shown that in the pathological process of KD, the NF-κB signaling system regulates transcription of almost all genes involved in inflammatory mediators and cell proliferation and activation. Activation of NF-κB signaling pathway is linked to the occurrence of KD vasculitis in the acute phase, which is likely to aggravate KD vasculitis response and participate in the formation of coronary artery injury [27]. ICAM-1 is considered an initiator of inflammatory cell adhesion and is also closely related to endothelial dysfunction [28], playing an important role in the development of cardiovascular disease [29, 30]. After endothelial injury, ICAM-1 expression increases, aggravating vascular injury by releasing more cytokines and chemokines [31]. These findings are consistent with the network-pharmacologic outcomes of this study and these target genes could be potential candidates for KD.

In addition, the GO enrichment analysis in this study showed that reactions to bacteria-derived molecules, reactions to lipopolysaccharides, and apoptotic processes are the major biological processes for CR treatment of KD. The disturbance of these biological processes is likely the cause of KD vasculitis and suggests that CR may play a protective role in blood vessels by improving these biological processes. Accordingly, CR may then be important in the regulation of different biological functions of KD.

Finally, the enrichment analysis of KEGG pathway found that the four signaling pathways closely related to CR and KD prevention and treatment included AGE-RAGE signaling pathway, fluid shear stress and atherosclerosis, TNF-signaling pathway and Toll1-like receptor signaling pathway. Since RAGE is a cell surface molecule of the immunoglobulin superfamily, a high expression of it on the surface of circulating endothelial cells in KD children is associated with the occurrence of coronary complications [32]. Also, Toll1-like receptor signaling pathway is a family of receptors composed of members of multiple receptors and has a key function when it comes to inflammatory and immune injuries of endothelial cells caused by pathogen infection and immune response [33]. Clinical studies have been helpful to confirm the correlation between TLR signaling pathway and inflammatory immune injuries of vascular endothelial cells in Kawasaki disease [34].

This study systematically explored the putative bioactive compounds in CR and pharmacological targets of CR for KD prevention and treatment through network pharmacology. All the ingredients of CR were extracted edible plants, and they were all claimed safe in previous applications. Therefore, this study provides a new way to explore the mechanism of CR in treating KD. However, the examinations here conducted are mainly based on the network pharmacology and bioinformatics database data, and has certain limitations. It is suggested for the future that relevant experiments are carried out to better explain the mechanisms of CR in the treatment of KD.

Conclusion

After conducting this examination, we postulated that BBR is effective in the treatment of young children with KD and can be considered as an alternative treatment for KD. This study has preliminarily revealed the possible mechanism of CR in the treatment of this disease by regulating multi-targets with multi-components. Furthermore, the outcomes demonstrated that a network pharmacology-based approach was useful for elucidation of the interrelationship between complex diseases and interventions of Chinese herbal medicines, potentially providing references for further research on its mechanisms of action in the future.

Methods

Patients

The sample group of this study is formed by 58 children with KD admitted to the Department of Cardiology, Shenzhen Children's Hospital from October 2018 to May 2019. They were randomly divided them into the routine treatment group and the routine treatment + BBR group. All children were typical KD patients and the diagnostic criteria were in line with the AHA and American Academy of Pediatrics 2004 guidelines [35].After divided, the kids were treated with routine treatment or the combination of routine treatment and BBR (10mg/kg, per time, bid) via oral. Three days after treatment, hematologic examination and IVIG resistance rate were compared between two groups.

Collection of chemical components in CR

The chemical components were all collected from Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database (http://lsp.nwu.edu.cn/tcmsp.php)[36], a system pharmacology platform designed for studying TCMs comprehensively. In this process, drug-likeness (DL) values ≥0.18, oral bioavailability (OB) ≥30% and blood-brain barrier (BBB) ≥−0.3 were selected as the active ingredients for the next step [37]. In addition, the compounds with high contents and significant pharmacological activities that did not meet the requirements were also retained.

KD target database building

Information on KD-associated target genes was collected from the following resources, including Drugbank Database (https://www.drugbank.ca/) [38], GeneCardsDatabase (https://www.genecards.org/) [39] and DisGeNET Database (http://www.disgenet.org/home/) [40]. It is noteworthy that the repetitive genes collected from the two sources were removed.

Target fishing for CR

The active ingredients of drugs play an important role in related biological functions via targets. The target fishing was used to search for or predict the potential targets of small molecules and the small molecular structure information of the active ingredients in CR by TCMS could also be predicted by

applying the Similarity Ensemble Approach [41] (SEA, http://sea.bkslab.org/), STITCH [42] (http://stitch.embl.de/) and Swiss Target Prediction [43] (Swiss, http://www.swisstargetprediction.ch/).

Construction and analysis of the pharmacological networks

In this phase, the CR targets and the acquired Kawasaki disease targets were screened by the online tools Venny2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) to draw the Venny map and also to obtain the common target genes of the intersection Then, the online tool STRING (http://string-db.org) was used to construct the target protein- protein interaction (PPI) network of CR acting on KD. The network construction was established, first, with a network between active compounds and targets of CR and also with PPI network of compounds and targets developed by linking the compound targets and predicted targets of other human proteins. Another PPI network of KD targets was also constructed by linking the known KD-related target.

Firstly, network between active compounds and targets of CR was constructed. Secondly, a PPI network of compounds and targets was developed by linking the compound targets and predicted targets of other human proteins. Thirdly, a PPI network of KD targets was constructed by linking the known KD-related targets and predicted targets of other human proteins. Finally, we constructed the intersection of the PPI network of compounds and KD. After that, the matrix analysis software Cytoscape3.6.0 (http://www.cytoscape.org/) was used to visualize all the correlations. The major ingredients and targets were represented by significant node, and the interactions were encoded by edges.

Gene Ontology (GO) and pathway enrichment analysis

To investigate the functional annotation and involved pathways of genes, the Gene Ontology (GO) database (http://geneontology.org/) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/) were calculated and evaluated by the Database for Annotation, Visualization and Integrated Discovery (WebGestalt, http://www.webgestalt.org) [44]. To match the purposes of this study, the difference was considered to be statistically significant at $p<0.05$.

Statistical analysis

Here, all quantitative data are presented as means ± SEM. For pair wise comparison of 2 or more groups of quantitative data, p values were calculated by t test and chi-square, as appropriate. $P<0.05$ was regarded as having statistical significance.

Abbreviations

CR CoptidisRhizoma; KD Kawasaki Disease; GO Gene Ontology; KEGG Kyoto encyclopedia of genes and genomes; PPI protein- protein interaction; AKT1 serine/threonine kinase 1; MAPK mitogen-activated protein kinase; PTGS2 prostaglandin-endoperoxide synthase 2; CASP3 apoptosis-related cysteine peptidase; TP53 tumor protein 53; MTOR mechanistic target of rapamycin; SRC sarcoma; ICAM1

intercellular adhesion molecule 1; NOS2/3 nielsen-symphonies; CRP c-reactive protein; NLRneutrophils/lymphocytes, PLR platelets/lymphocytes.

Declarations

Ethical Approval and Consent to participate

The research protocol was approved by the Ethics Committee of Shenzhen Children's Hospital (NO.20180601 and 202003802). Written informed consent was obtained from each patient included in the study.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

All data are available in the manuscript and they are showed in figures, tables and supplementary files.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This study was supported by National Nature Science Foundation of China (81870364) and Shenzhen Sanming Project (SZSM20162057) and Shenzhen Scientific Plan (JCYJ20190809164004023).

Authors' contributions

Mingguo Xu conceived and designed the research, reviewed and revised the manuscript. Xue Fan performed a preliminary analysis. Xue Fan and Xin Guo wrote the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

We thank Qian Gan for providing us room and laptop for shaping the paper.

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Table

Table 1
The hub genes of CR for KD treatment

Gene names	Description	Degree
AKT1	AKT serine/threonine kinase 1	30
CASP3	caspase 3	28
TP53	tumor protein p53	26
MAPK3	mitogen-activated protein kinase 3	26
PTGS2	prostaglandin-endoperoxide synthase 2	26
SRC	SRC proto-oncogene, non-receptor tyrosine kinase	25
MAPK8	mitogen-activated protein kinase 8	23
MAPK14	mitogen-activated protein kinase 14	23
RELA	RELA proto-oncogene, NF-kB subunit	23
MTOR	mechanistic target of rapamycin	23

Figures

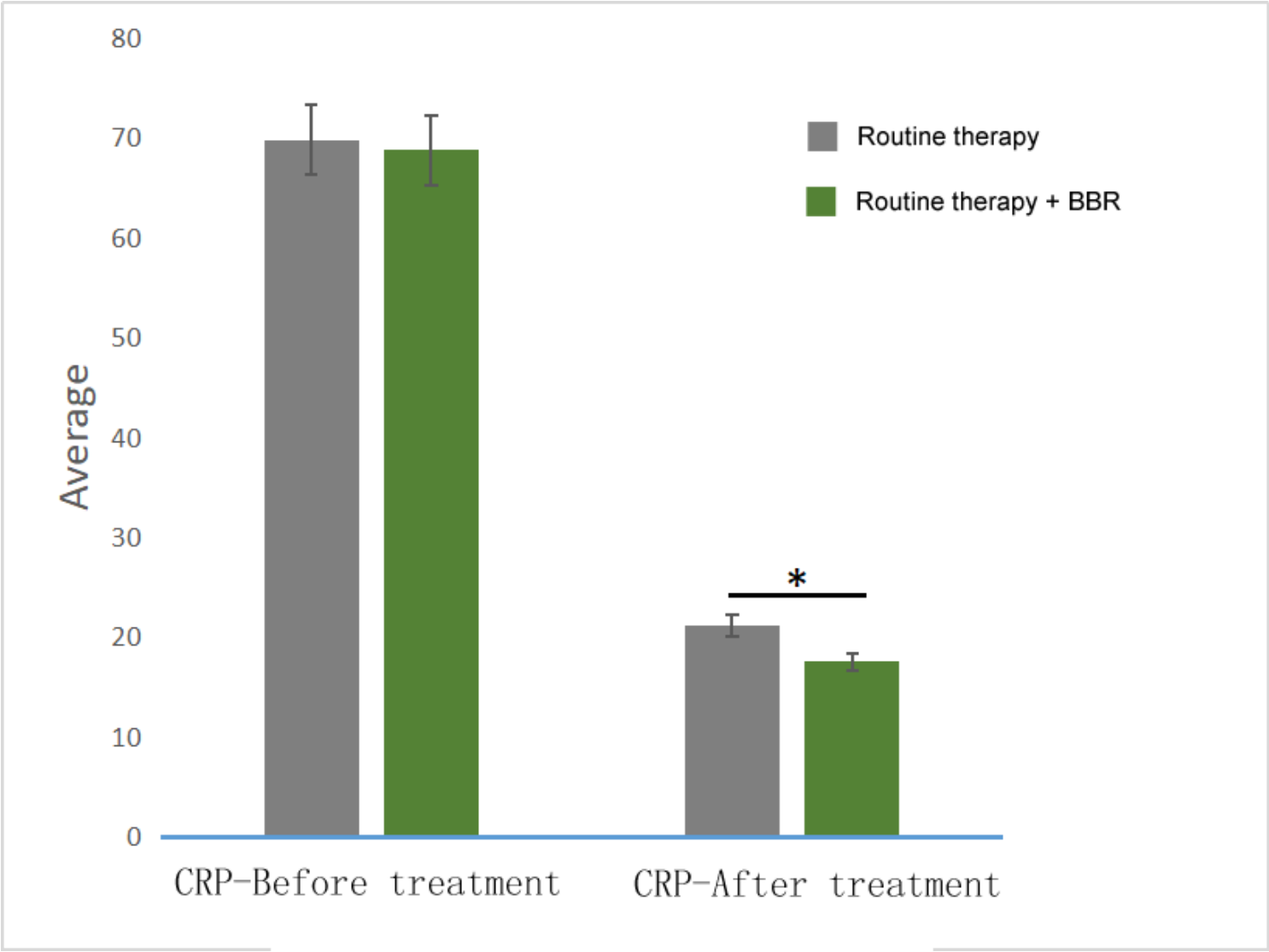

Figure 1

Changes in the average value of CRP before and after treatment between the routine group and the combined group.

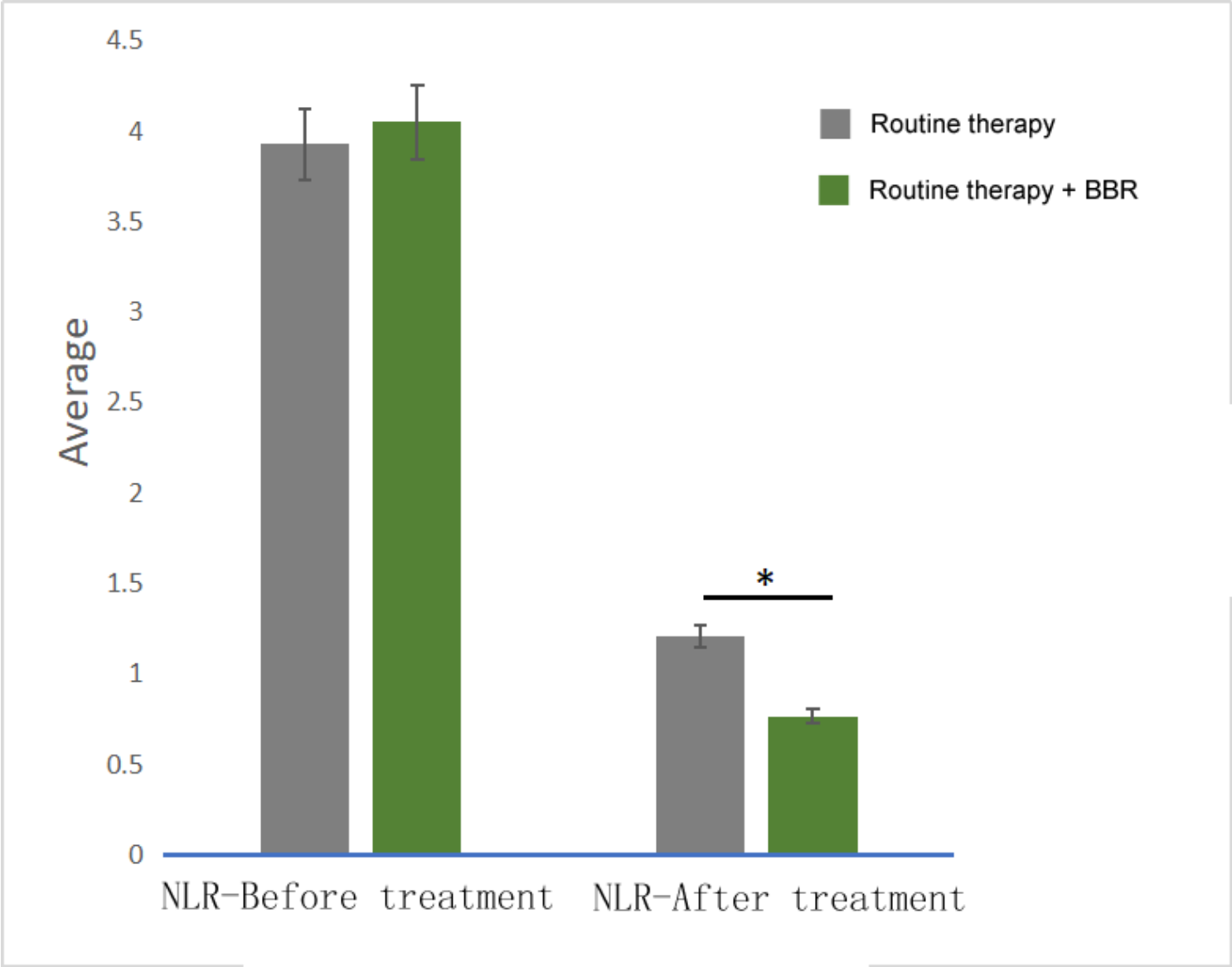

Figure 2

Changes in the average value of NLR before and after treatment between the routine group and the combined group.

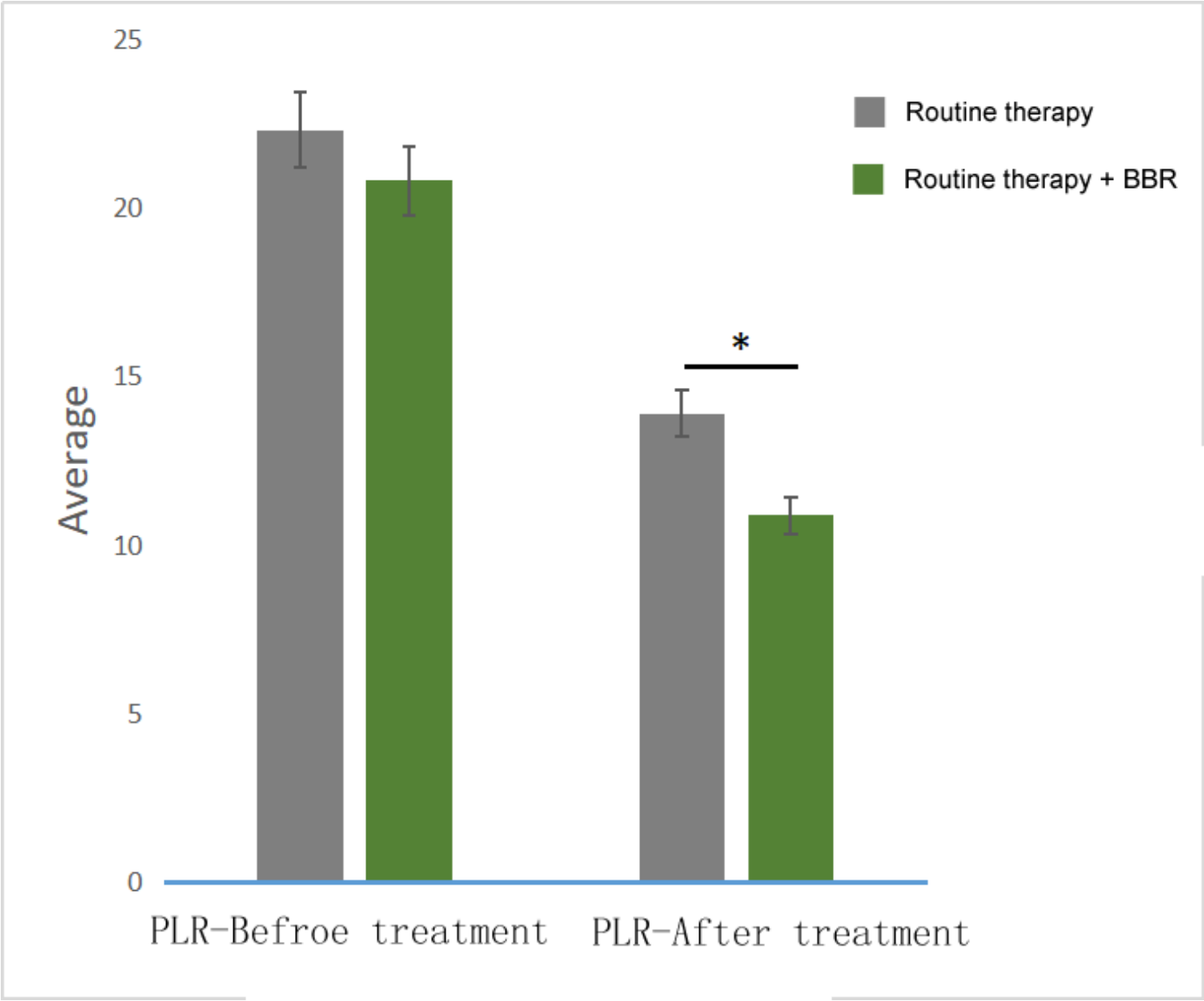

Figure 3

Changes in the average value of PLR before and after treatment between the routine group and the combined group.

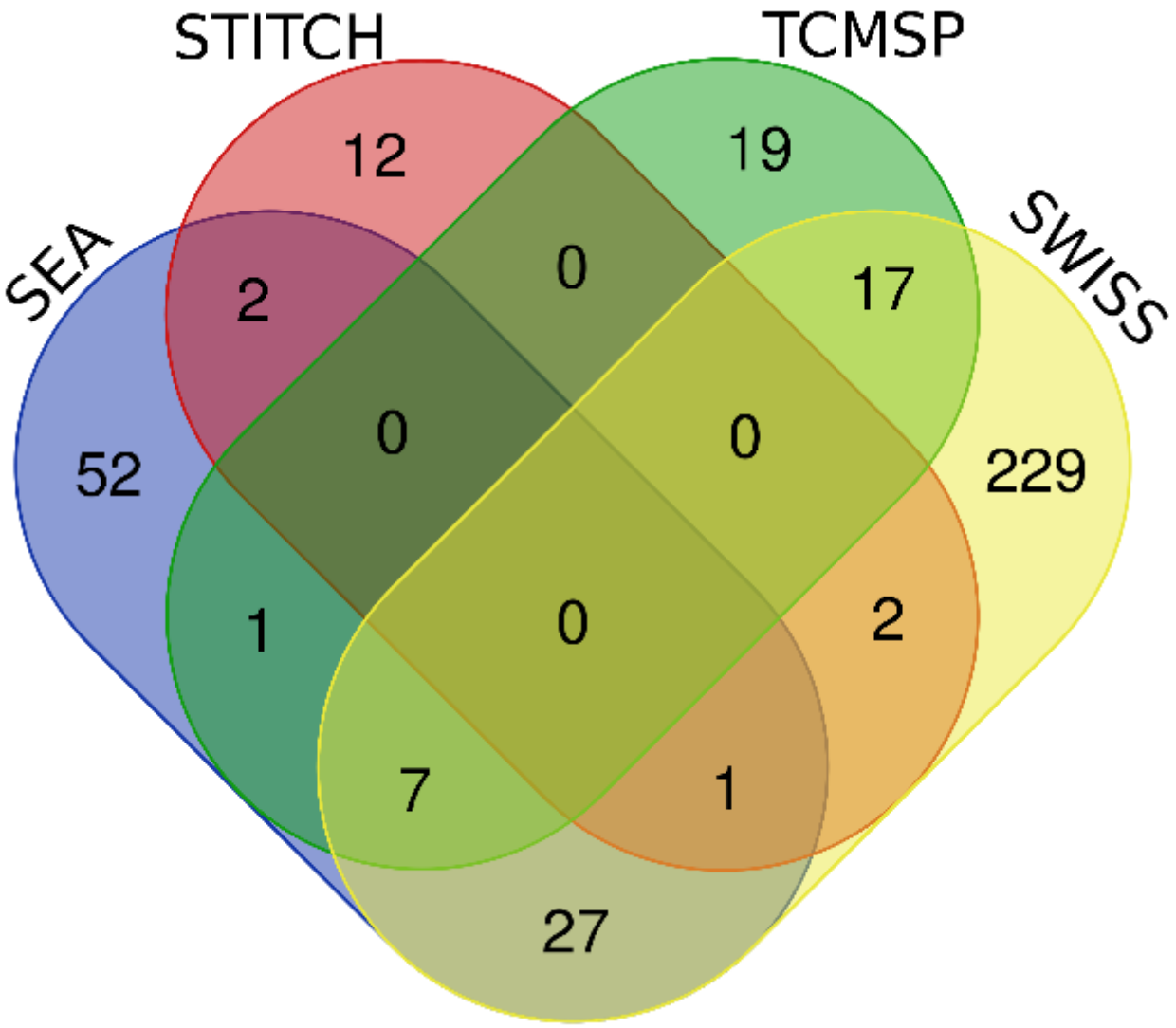

Figure 4

Target genes of CR from TCMSP、SWISS、SEA、STITCH databases.

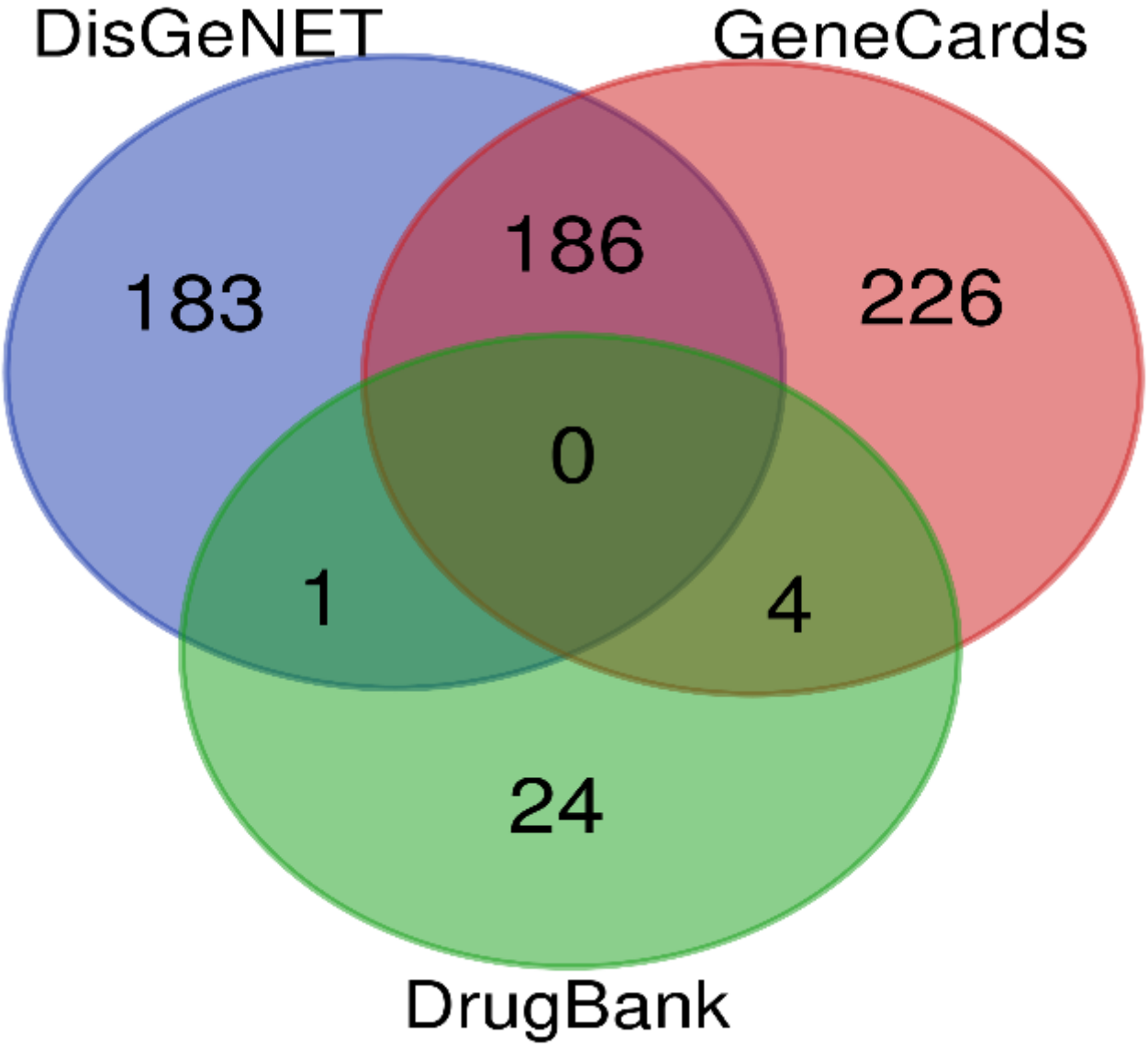

Figure 5

Target genes of KD from DisGeNET、DrugBank、GeneCards databases.

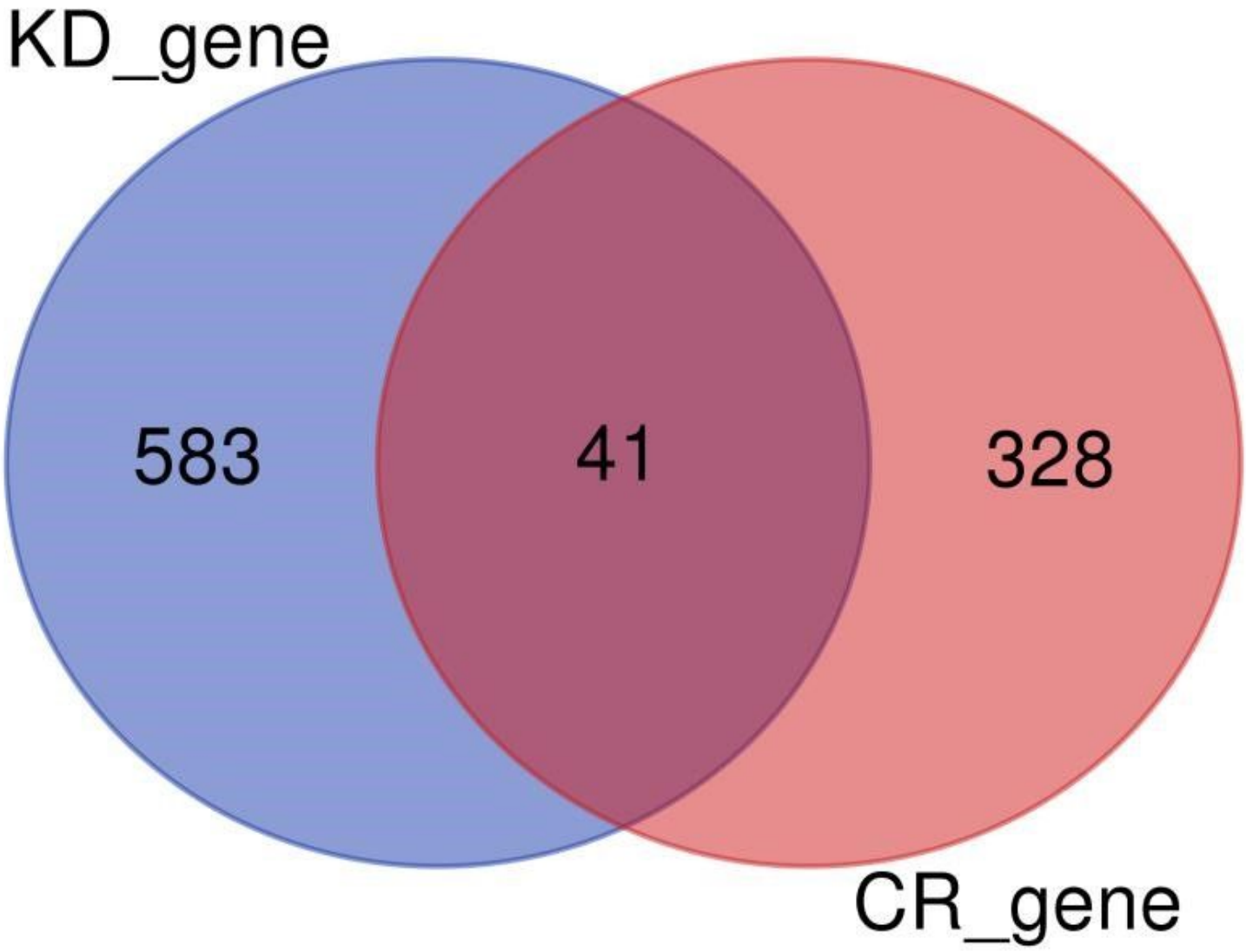

Figure 6

Venn diagram of the intersection of CR gene and KD gene.

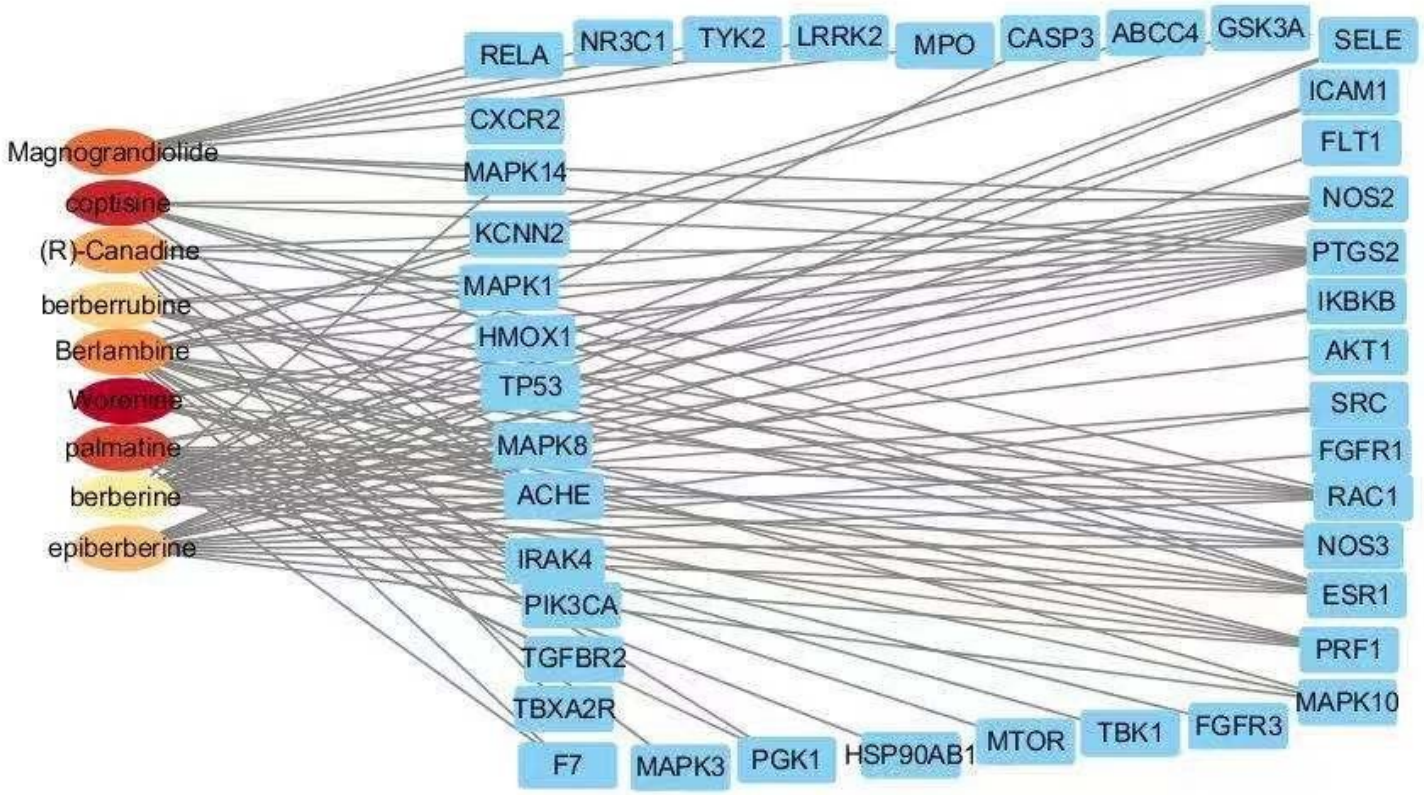

Figure 7

Compound–target genes network. Red circles represent active ingredients in CR. Blue rectangular represent targets of CR. Edges represent interaction between ingredients and targets.

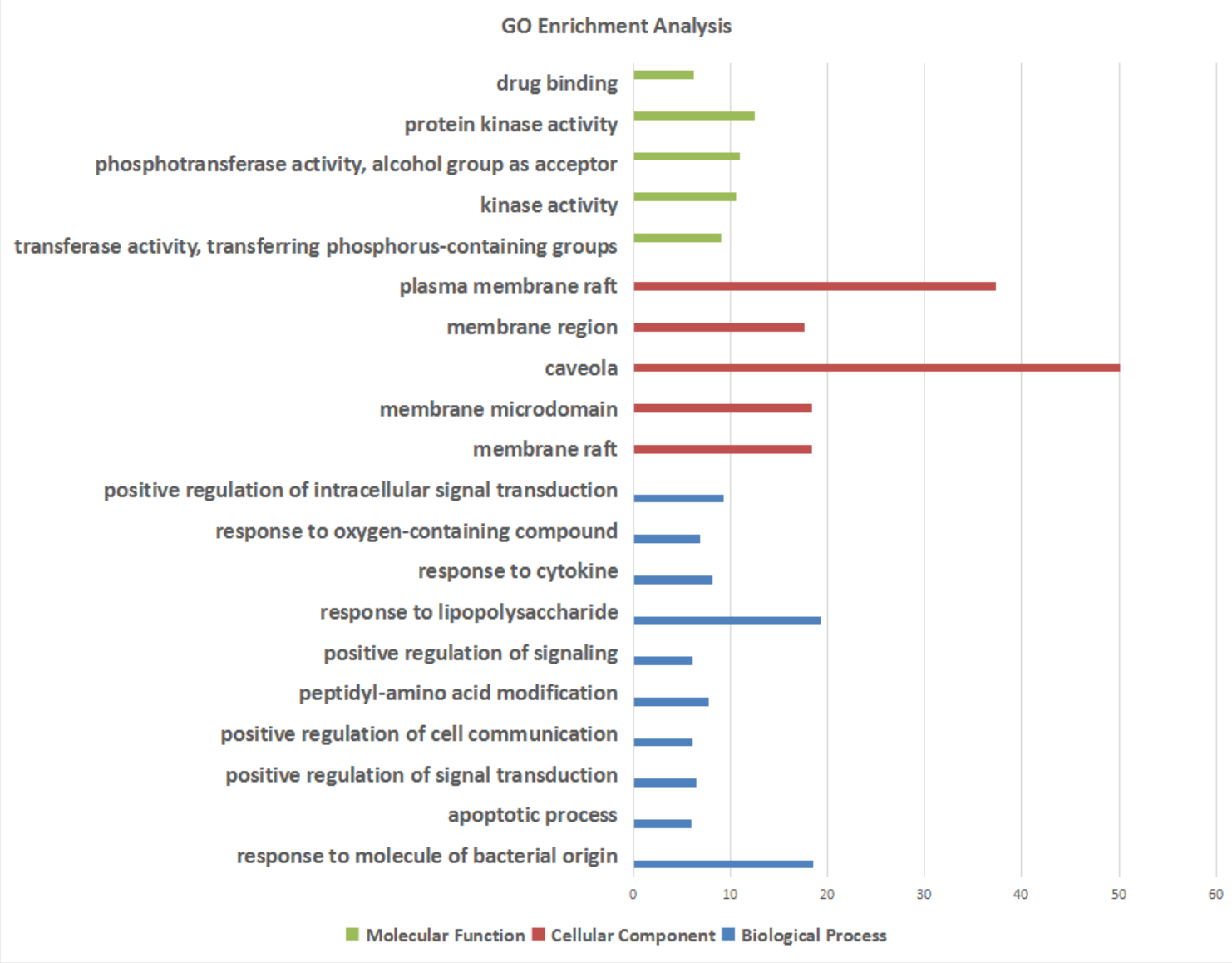

Figure 8

GO analysis of the candidate direct targets of CR for KD. Functionally grouped network of enriched categories was generated for the target genes. The x-axis represents the enrichment scores of these terms ($p \leq 0.05$); the y-axis represents significantly enriched BP□CC and MF categories.

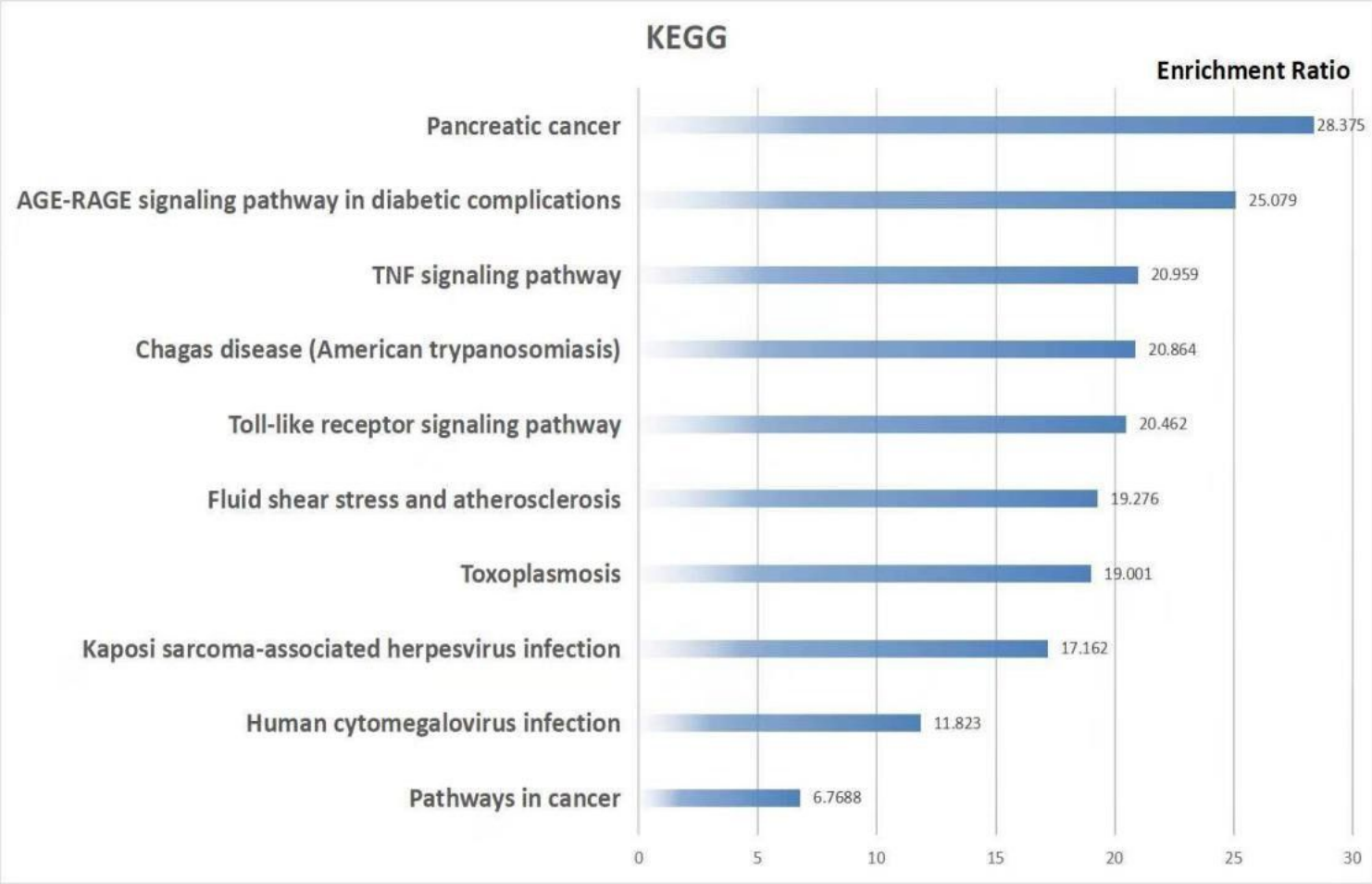

Figure 9

The KEGG pathway annotation of the candidate direct targets of CR for KD treatment.

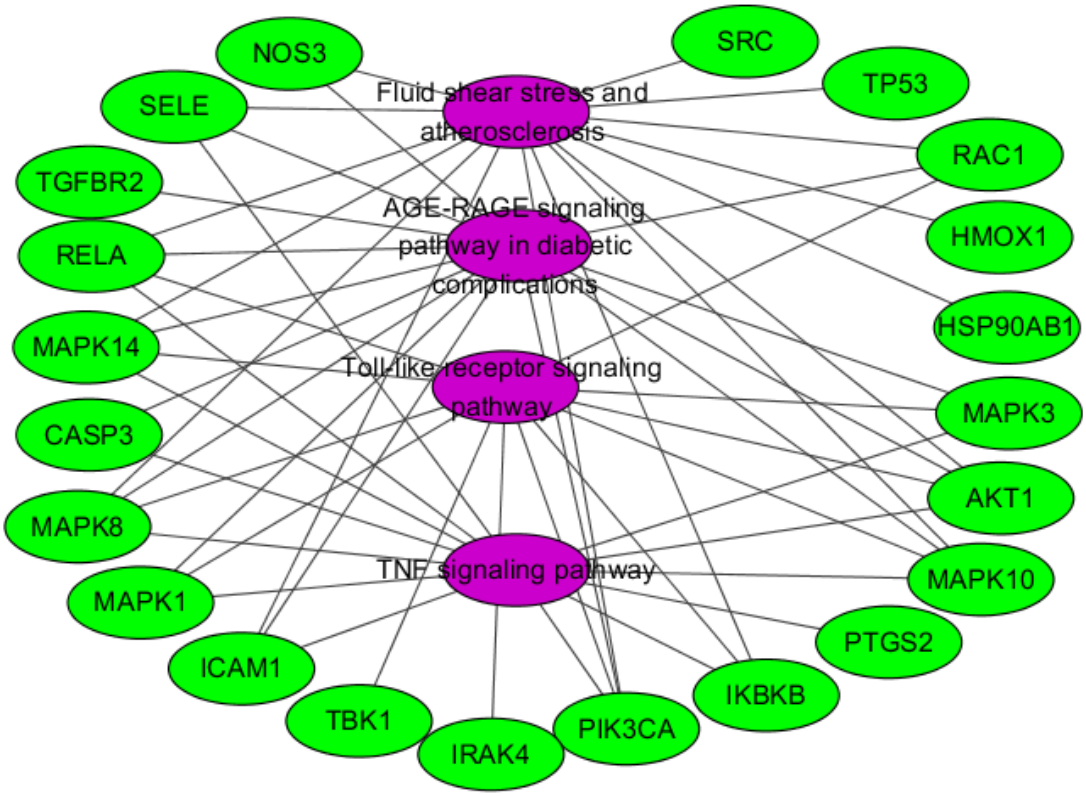

Figure 10

The signaling pathway diagram of the candidate direct targets of CR for KD treatment.

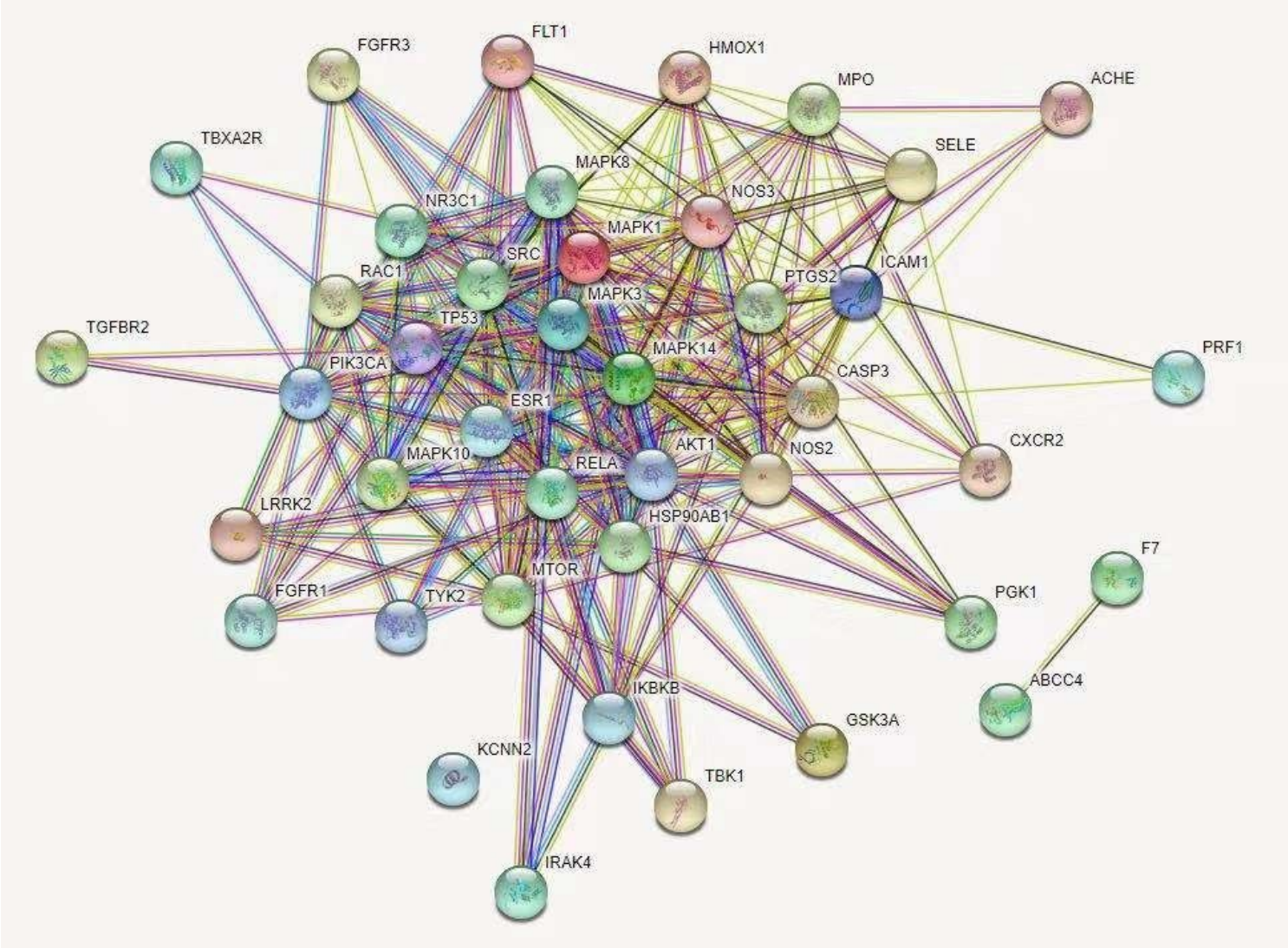

Figure 11

Interaction network of all compound-target genes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile2.xlsx
- Supplementaryfile1.xlsx