

Study on the Mechanism of Bushen Jianpi Decoction in the Treatment of T2DM Based on Network Pharmacology

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Research

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Abstract

Through the preliminary clinical observation, we had found that Bushen Jianpi decoction (BJD) combined with had better efficacy and less side effects, but its mechanism was not clear. The purpose of this study was to determine its molecular targets and mechanism in T2DM therapy by means of network pharmacology and molecular docking. Results: A total of 144 candidate compounds and 1103 differentially expressed genes were screened, and 43 common targets related to T2DM in BJD were identified. The "TCM-compound-target-disease" network suggested that quercetin, luteolin and kaempferol were the top three compounds. Through protein-protein interaction network, 45 core target genes were identified, including ITGA4, TP53, MYC and so on. GO enrichment showed that genes were significantly enriched in biological processes such as response to oxidative stress, response to lipopolysaccharide, response to molecule of bacterial origin and response to reactive oxygen species. KEGG enrichment showed that there was significant gene enrichment in Fluid shear stress and atherosclerosis, TNF signaling pathway, P13K-Akt signaling pathway, IL-17 signaling pathway and AGE-RAGE signaling pathway in diabetic complications signal pathways. The results of molecular docking showed that the key components of BJD had good binding potential with target genes. Conclusions: BJD may play a role in the treatment of T2DM through anti-inflammation, antioxidation and regulating metabolism, but it still needs to be further confirmed by experiments. Keywords: Network pharmacology, GEO database, Molecular docking, Bushen Jianpi decoction; T2DM

Background

Type 2 diabetes mellitus (T2DM) is an endocrine-metabolic disease characterized by hyperglycemia, which is caused by the decrease of insulin secretion (or relative decrease) caused by insulin resistance (IR) accompanied by islet β -cell dysfunction. According to the statistics of the International Diabetes Federation (IDF) [1], the number of people with diabetes in China reached 116 million in 2019, ranking first in the world. About 820000 people / year died of diabetes and its complications. IDF predicts that by 2045, the number of diabetics in the world will reach 700 million, while the number of diabetics in China will reach 151 million. At present, the main clinical drugs for the treatment of T2DM are insulin and its analogues, glinids, sulfonylureas and so on. These drugs show a variety of side effects such as neurotoxicity, cardiotoxicity and other damage to the health of patients [2], such as metformin can cause dyspepsia, rosiglitazone has potential side effects on cardiovascular system [3]. T2DM belongs to the category of "eliminating thirst" in TCM. Compared with western medicine, TCM emphasizes holistic thinking, curing both symptoms and root causes, controlling blood sugar and effectively alleviating the occurrence of complications, which help to improve the quality of life of patients. Through the preliminary clinical observation in our group, it was found that compared with the western medicine treatment group, the BJD

treatment group not only effectively reduced fasting blood glucose (FPG), 2-hour postprandial blood glucose (2hPG), insulin resistance index (Homa-IR) and islet function index (Homa-islet), but also had lower adverse reactions (nausea, vomiting, diarrhea), but its mechanism was not clear. Based on systems

biology and bioinformatics, network pharmacology constructs a multi-molecular, multi-target and multi-link network model of "drug-target-disease", which can effectively predict the efficacy and mechanism of drugs. From an overall point of view, this discipline caters to the new trend of systematic research on compound prescriptions of TCM in the big data era. Therefore, this study intends to explore the mechanism of BJD in the treatment of T2DM through network pharmacology technology, and to explain the coordination of multi-components, multi-targets and multi-links in the treatment of diseases by means of modern science and technology, so as to provide new ideas and basis for its clinical application and experimental research.

Materials And Methods

The active components of BJD were searched by Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform(TCMSP), and their corresponding molecular targets were determined by UniProt database. The differentially expressed genes of T2DM patients and healthy people were compared by GEO database, and the target genes related to T2DM were screened(GSE25724, GSE29221, GSE29226 and GSE29231). Then, the possible targets of BJD were intersected with the differentially expressed genes of T2DM, and the common targets of BJD in the treatment of T2DM were obtained by limma package, which were used to establish protein-protein interaction (PPI) network, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis by clusterProfiler and pathview package. After screening the key active components and target genes, the AutoDock software was used for molecular docking to verify its binding activity.

Table 1
Binding energies of BJD's key components to the target gene molecules

	quercetin	luteolin	kaempferol	apigenin	beta-sitosterol
JUN	-3.46	-2.94	-3.02	-3.35	-4.87
FOS	-5.41	-4.99	-4.88	-5.31	-6.05
IL6	-3.59	-4.99	-4.93	-4.70	-5.53
BAX	-5.31	-5.53	-5.35	-6.23	-6.59
MYC	-3.43	-3.85	-3.50	-4.54	-3.90

Results

Active ingredient

Through screening the TCMSP database, 144 qualified compounds were selected as candidate compounds. At the same time, the raw data of GSE25724, GSE29221, GSE29226 and GSE29231 were standardized and batch effects were removed. Differentially expressed genes were obtained by limma

package and volcanic map was drawn to better show the distribution of genes. Among them, there are red up-regulation and blue down-regulation (Fig. 1). A total of 1103 differentially expressed genes were obtained, including 313 up-regulated genes and 790 down-regulated genes.

TCM-complex-target-disease network

Through the intersection of BJD prediction targets with T2DM differentially expressed genes, 96 BJD-T2DM related complexes and 43 common targets were identified. A TCM-complex-target-disease network (Fig. 2) was built using Cytoscape3.8.0 software. The network consists of 149 nodes (96 compounds, 43 targets, 8 herbs and 2 T2DM gene difference types) and 345 edges. Through the "Analyze Network" function, we got the Degree centrality (DC) corresponding to each node in the network. The higher the DC, the more central the node. Sorted by DC, quercetin (35), luteolin (13) and kaempferol (10) were the top three compounds, which were related to 32, 11 and 9 target genes, respectively. Because of the important position of these three compounds in the network, they may be the key living components of BJD.

The PPI network analysis

PPI network is the relationship network of biomolecules, which plays an important role in the biological process and helps to understand the mechanism of biological activity and the pathogenesis of disease. As shown in Fig. 3 (a), the PPI network consisted of 2710 nodes and 84831 edges, representing 2710 interacting proteins and 84831 interactions. The targets with $DC \geq 89$ (2 times median DC) were screened, and a PPI network composed of 644 nodes and 21257 edges was constructed. (Fig. 3(b)), in this network, the median parameters of EC, LAC, BC, CC and NC were 0.029526511, 14.75, 404.2932, 0.518548 and 16.89873, respectively. The above data could be used as a new reference standard for further screening, and further determined to meet the targets of $EC \geq 0.029526511$, $Lac \geq 14.75$, $BC \geq 404.2932$, $CC \geq 0.518548$ and $NC \geq 16.89873$ at the same time (Fig. 3(c)). On this basis, the top 45 were selected as the core target (Fig. 3(d)), including ITGA4, TP53, MYC. These genes may play a hypoglycemic effect through direct and indirect effects.

GO and KEGG analysis

43 common targets were used for GO and KEGG pathway analysis, which were realized by clusterProfiler package. GO enrichment analysis was carried out from three aspects: biological process (BP), cell composition (CC) and molecular function (MF). There were 225 enrichment GO items with $FDR \leq 0.05$ and $q \leq 0.05$. Among them, there were 132 biological process items, 53 cellular components items and 40 molecular function items. The results showed that, BP (Fig. 4) was mainly related to response to oxidative stress, response to lipopolysaccharide, response to molecule of bacterial origin and response to reactive oxygen species; CC (Fig. 5) was mainly related to membrane raft, membrane microdomain, membrane region and organelle outer membrane; MF (Fig. 6) was mainly related to cytokine activity, receptor ligand activity, signaling receptor activator activity and cytokine receptor binding. According to the results of KEGG enrichment (Fig. 7), the mechanism of BJD in the treatment of T2DM is mainly concentrated in Fluid shear stress and atherosclerosis, TNF signaling pathway, P13K-Akt signaling pathway, IL-17 signaling pathway and AGE-RAGE signaling pathway in diabetic complications, and the

genes most related to the most pathways were JUN, CHUK, FOS, IL6 and BAX. Pathview package [10] was used to visualize the enrichment of Fluid shear stress and atherosclerosis and AGE-RAGE signaling pathway in diabetic complications respectively (Fig. 8, Fig. 9).

Molecular docking

Molecular docking is a technique that simulates the interaction between receptor protein macromolecules and small ligand molecules. The affinity of two counterparts is predicted by calculating the binding energy between them. When the binding energy is less than 0, it suggests that the two molecules bind spontaneously, and the smaller the binding energy is, the more stable the conformation is. The results showed that most of the components in BJD could well bind to the target genes (Table 1). FOS, BAX, IL6 and MYC could dock well with most of the active components. Figure 10 illustrated some of the local structures of molecular docking in detail.

Conclusion

T2DM is an endocrine disease characterized by metabolic disorders and chronic complications, which is related to heredity, lifestyle, environment and other factors. The pathogenesis of T2DM is mainly related to insulin resistance (IR), islet β -cell dysfunction, inflammation and oxidative stress. Previous studies have shown that HJ, HQ, DH and other herbs have hypoglycemic effect [11]. Polygonatum polysaccharide, the main active compound in HJ, reduces blood sugar by inhibiting the activity of α -glucosidase, and has anti-inflammatory, anti-tumor and improving immune function [12]. Flavonoids from semen Astragali is the main extract of SYZ, which can enhance the antioxidant function of liver and improve the ability of tissue to scavenge free radicals, so as to relieve the damage of free radicals to islet β cells and promote the repair and regeneration of islet β cells, which help to achieve the role of regulating blood sugar [13,14]. Active compounds in DH can improve glucose metabolism and β -cell function, regulate intestinal microflora, and reduce oxidative stress and inflammation. However, the specific mechanism of BJD in the antidiabetic process is not clear. Therefore, we used the method of network pharmacology to clarify the mechanism of the active components of BJD on type 2 diabetes. In the case of hyperglycemia, the body interferes with the mitochondrial electron transport chain, up-regulates the polyol pathway, increases the self-oxidation of glucose, promotes the production of free radicals, induces oxidative stress, damages islet β cells, and induces insulin resistance [19]. There is a vicious circle among hyperglycemia, oxidative stress and diabetes [20]. At the same time, some studies have reported that T2DM is a low-grade inflammatory disease, and the inflammatory response mediated by related inflammatory factors can lead to the occurrence and development of T2DM. These suggest that the occurrence and development of diabetes is closely related to oxidative stress and inflammatory reaction. On the other hand, the drugs developed with antioxidant and anti-inflammatory as the starting point have achieved satisfactory results in the treatment of T2DM alone or in combination with traditional hypoglycemic drugs [23–25], which further proves the broad prospect of antioxidant and anti-inflammatory therapy in the field of T2DM. The results of this study showed that (Fig. 2), quercetin, luteolin and kaempferol were identified as the active components related to the most targets, while the

results of molecular docking (Table 1, Fig. 10) it is also confirmed that they have good binding properties to most target genes, suggesting that the above complexes may be the key components of BJD anti-T2DM. Quercetin is a flavonol compound which is widely distributed in nature and has a variety of biological activities. Quercetin is considered to be the most effective active oxygen (ROS) scavenger. Quercetin can not only improve lipid peroxidation, but also inhibit the production of several pro-inflammatory factors, such as tumor necrosis factor- α and carbon monoxide. At the same time, the antioxidant and anti-inflammatory effects of quercetin in T2DM treatment have been confirmed by various experiments in vivo and in vitro. Li [26] administered different doses of quercetin to diabetic rats and extracted the pancreas of diabetic rats. The study found that quercetin can effectively reduce the production of ROS in INS-1 cells and protect INS-1 cells from oxidative stress induced by high glucose. Moreover, quercetin reversed the expression of apoptotic proteins BAXm-RNA and PDX-1m-RNA and inhibited the apoptosis of INS-1 cells, which proved that quercetin could up-regulate the cell activity and insulin secretion of INS-1, and further improve blood glucose and glucose tolerance.

Luteolin, as a kind of natural weakly acidic tetrahydroxyflavonoids, has strong antioxidant and anti-inflammatory activities. In patients with T2DM, hyperglycemia down-regulates SIRT6-FOXO3a pathway and activates p47phox expression, and P47phox is necessary for ROS production [27]. KIMA [28] study found that in T2DM patients, luteolin-treated group significantly down-regulated p47phox gene, reduced the production of ROS; this study further found that under the condition of hyperglycemia, human monocyte superoxide dismutase increased, but decreased to a normal level in the presence of luteolin, which suggests that luteolin inhibits the production of ROS by activating the expression of SIRT6 and FOXO3a, to achieve the effect of prevention and treatment of diabetes. Kaempferol is also a natural flavonoid, and its anti-inflammatory, antioxidant and anticancer effects have been reported for the treatment of a variety of diseases, such as diabetes, obesity and cancer (such as liver, colon and skin cancer [29]). The above three compounds all have significant hypoglycemic effects on T2DM, and all have high oral availability (46.43%, 36.16%, 41.88%). They are considered to be representative compounds in BJD. As shown in Fig. 3, in order to obtain more accurate T2DM-related genes, we performed a series of parameters to screen the key goals in the PPI network, and finally identified 45 core goals, which may play an important role in the pathophysiological process of type 2 diabetes through direct or indirect action. Among them, the first five genes are MYC, TP53, APP, HSPB1 and EGFR. MYC is a highly pleiotropic transcription factor. It is conservatively estimated that 15% of all genes are directly regulated by MYC, including key roles in metabolism, cell cycle, differentiation and apoptosis. Increased expression of MYC protein can promote cell proliferation, however, this effect has been shown to be associated with a variety of human cancers [30]. Hofmann JW [31] studies have shown that MYC haploid deficiency (MYC $^{+/-}$) mice have longer lifespan, higher metabolic rate and healthier fat metabolism, suggesting that the expression of MYC is not conducive to health. In T2DM machine, high glucose suppresses neurogenic differentiation 1 (NEUROD1)-mediated insulin gene promoter by up-regulating the expression of MYC, thus affecting insulin gene transcription [32]. Not only that, MYC is also an oxidative stress response gene [33 ~ 34], which is thought to be related to islet β -cell dedifferentiation [35]. TP53 is one of the most closely related genes in human cancer. more and more evidence shows that it plays a

regulatory role in diabetes, obesity and other fields [36], as well as in non-cancerous diseases (such as T2DM). P53 is activated in response to various injuries including oxidative stress, resulting in islet β -cell death. In addition, p53 affects glucose metabolism [37–40] and induces T2DM by directly regulating glucose uptake, glycolysis and gluconeogenesis. In recent years, progress has been made in the study of Amyloidprecursorprotein (APP) in peripheral organ diseases except Alzheimer's disease. AnYA [41] study shows that the increased expression of APP in whiteadiposetissue will lead to chain effects such as weight gain, impaired glucose tolerance and insulin resistance. HSPB1 is a member of the small molecular heat shock protein family, which is closely related to diabetes [42]. The expression of HSPB1 is up-regulated under the action of cytokines and other cellular stressors to protect cells from oxidative stress, inflammation and apoptosis. It has been found that the islets of transgenic mice with high expression of HSPB1 are more resistant to β -cell apoptosis, indicating the correlation of HSPB1 in the protection of β -cells and islets [43]. EGFR is involved in the proliferation, apoptosis and invasion of tumor cells. EGFR and its ligands play an important role in regulating the number of pancreatic β cells, especially islet β cells. Therefore, the above genes may become valuable clinical biomarkers and provide a new direction for the diagnosis and treatment of T2DM.

In BP, the common target of), BJD anti-T2DM is mainly concentrated in response to oxidative stress, cellular response to oxidative stress, response to lipopolysaccharide, regulation of angiogenesis, smooth muscle cell proliferation and so on. Oxidative stress is the pathological process of tissue and organ damage caused by the imbalance between oxidation and antioxidant systems in the body. it is the common soil that catalyzes the activation of protein kinase C, polyol bypass, the formation of terminal glycation products, inflammation and other pathological links [44]. It is related to the occurrence and development of multi-system and chronic diseases such as diabetes, cardiovascular disease and so on. In recent years, it has been found that some antioxidant drugs, such as berberine, scavenge free radicals, inhibit the activation of polyol bypass and reduce the formation of terminal glycosylation products [45]. To promote insulin secretion and improve insulin resistance [46]. Therefore, BJD anti-T2DM may be related to antioxidation, and through antioxidation, regulation of angiogenesis and other ways to protect vascular endothelial function and prevent chronic complications of diabetes. In addition, BJD treatment of T2DM is also related to CC and MF, including mitochondria, endoplasmic reticulum, serine/threonineproteinkinasecomplex and cytokineactivity. Some studies have shown that there are ultrastructural abnormalities in the cells of patients with T2DM, such as endoplasmic reticulum dilation, mitochondrial swelling, cytoplasmic vacuolation, abnormal chromatin condensation and so on. In KEGG analysis, the first 20 KEGG pathways were screened, including Fluid shear stress and atherosclerosis, TNF signaling pathway, P13K-Akt signaling pathway, IL-17 signaling pathway and AGE-RAGE signaling pathway in diabetic complications, among which Fluid shear stress and atherosclerosis pathway was the most significant. In T2DM, insulin is the main ligand that regulates the metabolism of PI3K/AKT pathway. On the one hand, PI3KAkt signaling pathway increases glucose utilization and reduces gluconeogenesis in liver and muscle by activating glucose metabolism pathway; on the other hand, P13K/AKT-mediated growth factor signal plays an important role in glucose, lipid and protein metabolism [54]. Hyperglycemia, oxidative stress, polyol pathway and inflammation stimulate the production and

modification of AGEs[55–56]. The combination of AGEs and RAGE increases the production of ROS, leading to the upregulation of inflammation, the induction of oxidative damage and changes in cell metabolism [57–59], while oxidative stress can further stimulate the production of AGE [60]. The intervention of diabetes includes not only the control of blood sugar, but also the prevention and treatment of complications. Diabetic complications include retinopathy, neuropathy, nephropathy, atherosclerosis (macroangiopathy) and microangiopathy. There is evidence that vascular dysfunction (macroangiopathy and microangiopathy) is the basis of most chronic complications of diabetes [47]. Hyperglycemia triggers vascular complications through the following different mechanisms: direct toxicity of glucose, increased production of free radicals, accumulation of advanced glycation end products, and redox disorders induced by sorbitol pathway [48–49]. Moreover, fluid shear stress also plays an important role in the development of diabetic angiopathy [50–52]. It can be seen that Fluid shear stress and atherosclerosis and AGE-RAGE signaling pathway in diabetic complications play an important role in the occurrence and development of T2DM complications. In addition, it is generally believed that the occurrence of insulin resistance is a chronic inflammatory process, and the mechanism of IR is closely related to inflammation. Inflammatory factors can participate in insulin signaling pathway to interfere with other signal transduction, and can also interact with oxidative stress process to aggravate IR. This study confirmed by KEGG analysis that BJD may also play an anti-T2DM role by regulating IL-17 signaling pathway to mediate inflammatory response.

In summary, this study shows that the mechanism of the treatment of T2DM is related to its antioxidation, anti-inflammation, regulation of metabolism and so on. Quercetin, luteolin and kaempferol in BJD can regulate most of the related indexes of T2DM; three key genes screened by PPI network (ITGA4, TP53, MYC) may provide new ideas for T2DM therapy; BJD may regulate anti-T2DM function through biological processes such as response to oxidative stress, cellular response to oxidative stress, response to lipopolysaccharide, regulation of angiogenesis, smooth muscle cell proliferation and so on. The above functions are mainly realized through Fluid shear stress and atherosclerosis, TNF signaling pathway, P13KAkt signaling pathway, IL-17 signaling pathway and AGE-RAGE signaling pathway in diabetic complications. Because this study is based on the existing database information, there is a lack of experimental verification. Therefore, it is necessary to carry out follow-up experimental analysis to confirm the reliability of the results of this study.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Feng Liu was responsible for the conception and design. Dongqiang Luo and Ying Shao were responsible for the acquisition of data and the analysis and interpretation of data. Yong Sun and Shuntang Du were responsible for the writing, review the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors report no conflicts of interest.

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Figures

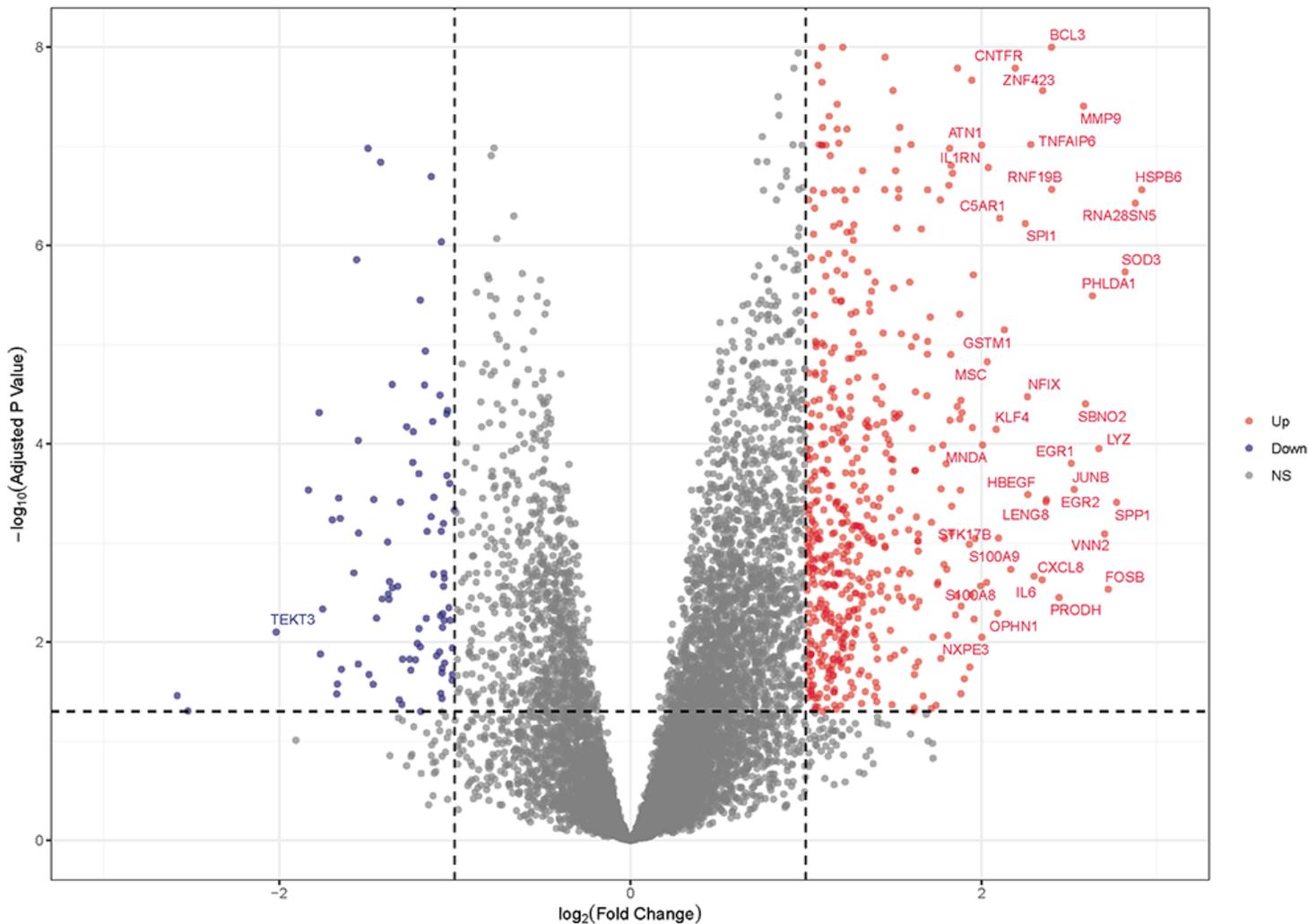


Figure 1

Gene volcano map shows the gene distribution in disease samples. Red and blue represent upregulated genes ($\log_2(\text{FC}) \geq 1$) and downregulated genes ($\log_2(\text{FC}) \leq -1$), respectively, whereas gray indicates no significant difference.

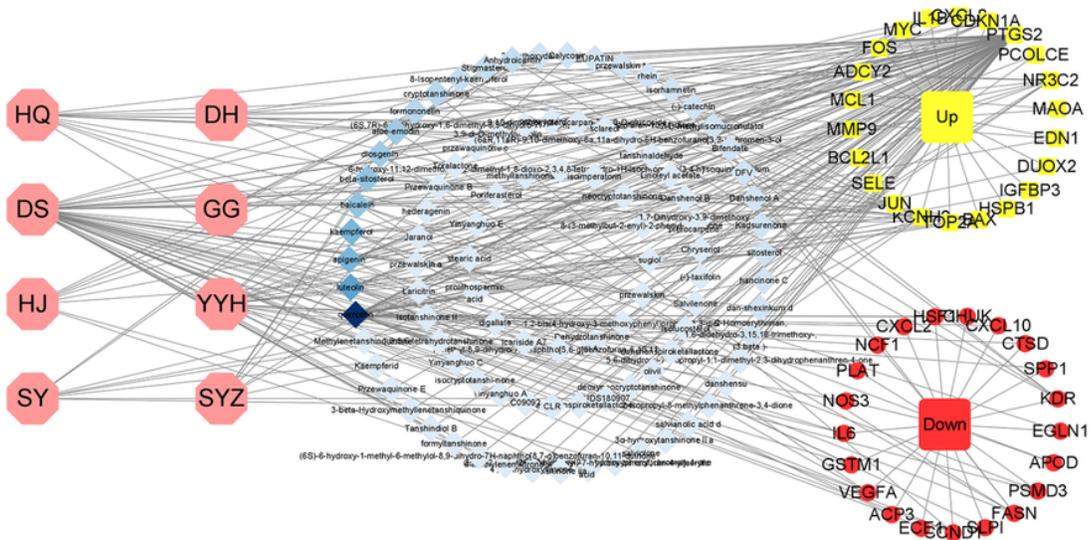


Figure 2

TCM-compound-targets-disease regulatory network. This network shows the targeted relationship among the active components of TCM, the intersection genes and disease. Pink nodes represent medicinal herb; YYH, HJ, HQ, SY, DS, DH, GG stand for Herba Epimedii, Rhizoma Polygonatum, Radix Astragali, Chinese yam, Radix Salviae Miltiorrhizae, Rhubarb and Pueraria lobata respectively. Blue nodes represent compound, the darker the color, the higher the DC value. Yellow nodes represent downregulated target genes, Red represent upregulated target genes.

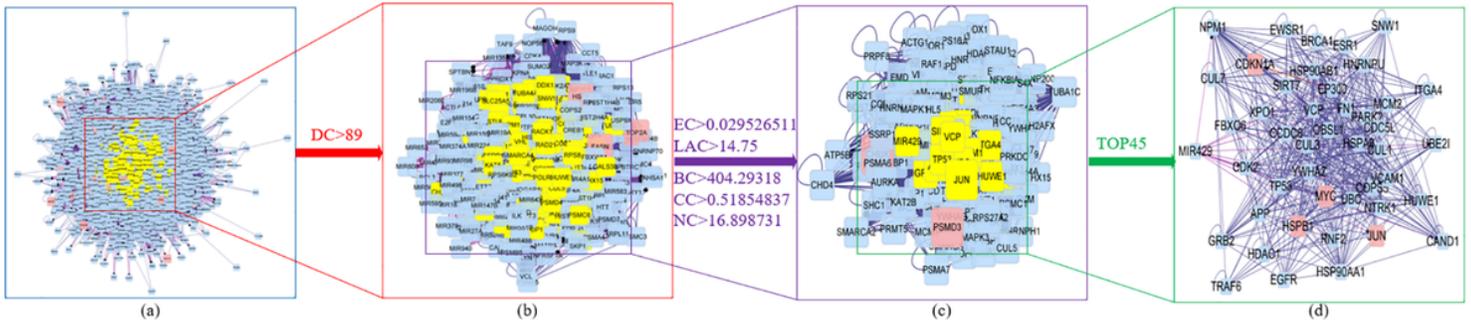


Figure 3

PPI Network and Topological Analysis. Topological analysis of the protein-protein interaction network. Herein, 2710 protein nodes were obtained according to the intersection genes. After screening by $DC \geq 61$ for the first time, a total of 644 protein nodes were obtained, and the 234 proteins were extracted according to EC, LAC, BC, CC, NC for the second time. Finally, the first 45 proteins were

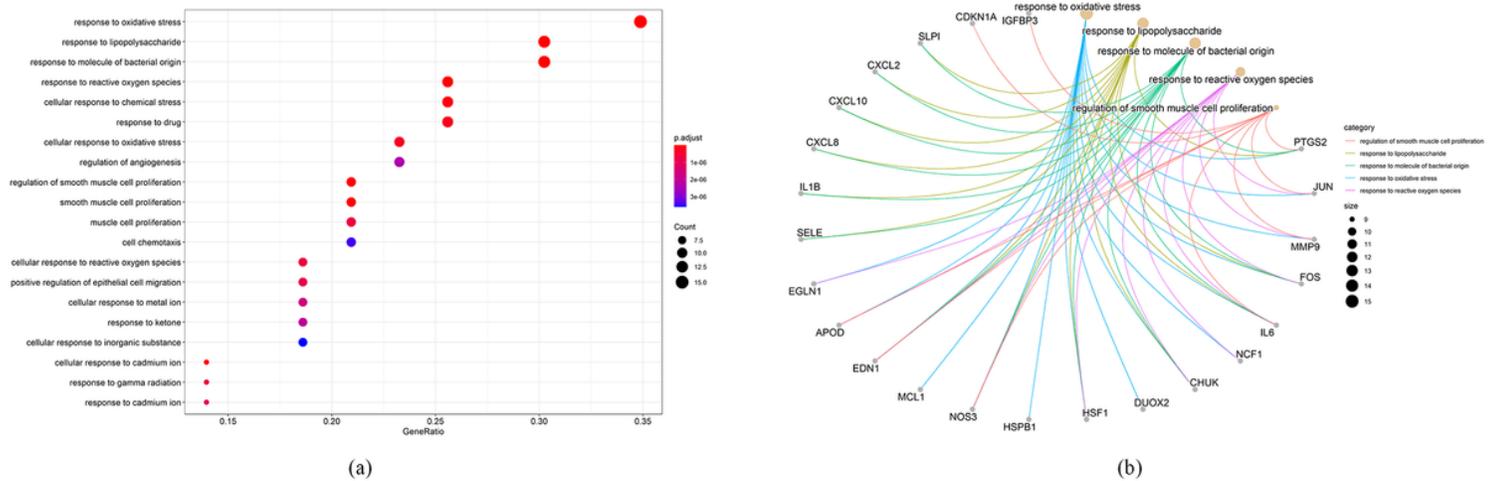


Figure 4

BP. (a) dotplot. The horizontal axis represents the gene proportion enriched in each entry, and the vertical axis shows the enrichment degree according to the corrected P value.; (b) cnetplot. The edge represents the gene enrichment on the BP.

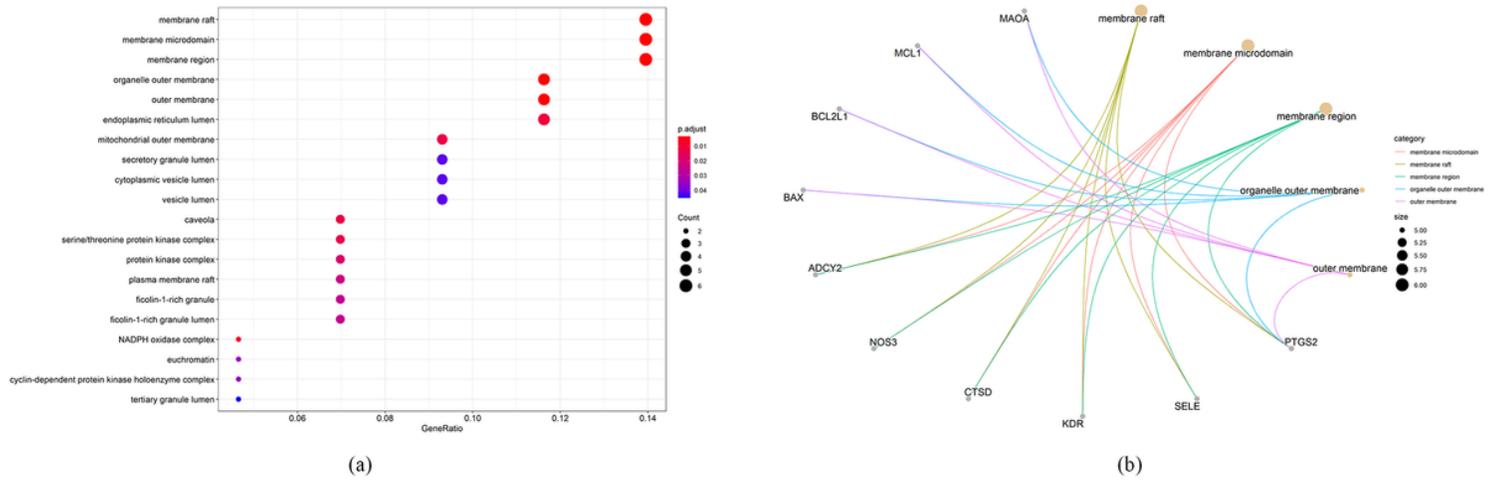


Figure 5

CC.(A)dotplot. The horizontal axis represents the gene proportion enriched in each entry, and the vertical axis shows the enrichment degree according to the corrected P value.; (b)cnetplot. The edge represents the gene enrichment on the CC.

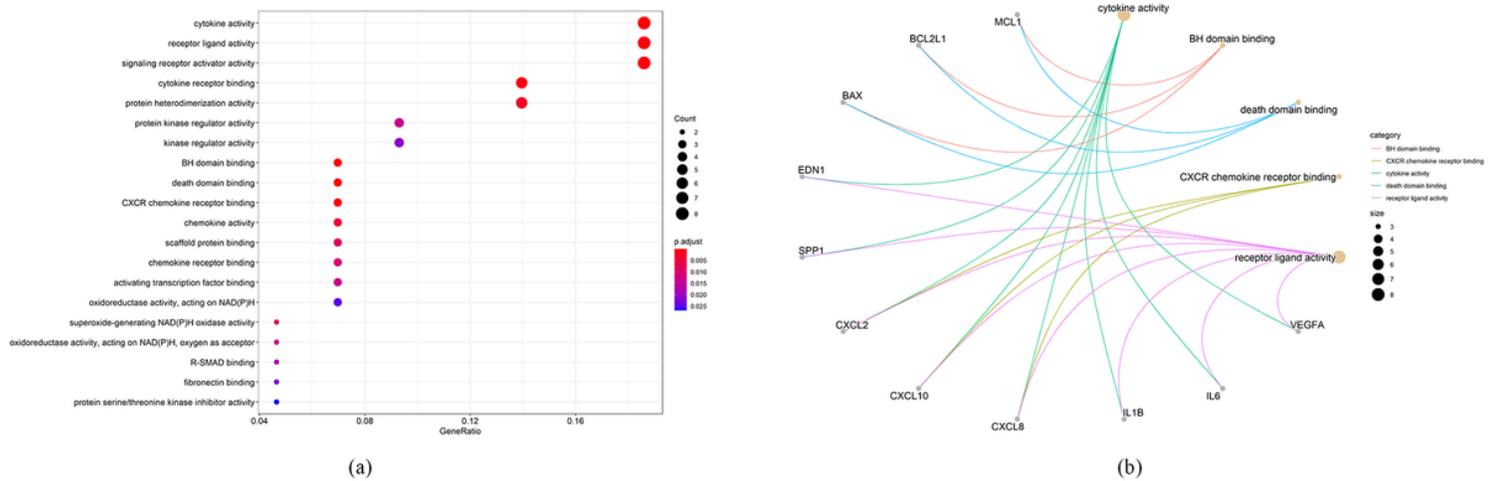


Figure 6

MF.(A)dotplot. The horizontal axis represents the gene proportion enriched in each entry, and the vertical axis shows the enrichment degree according to the corrected P value.; (b)cnetplot. The edge represents the gene enrichment on the MF.

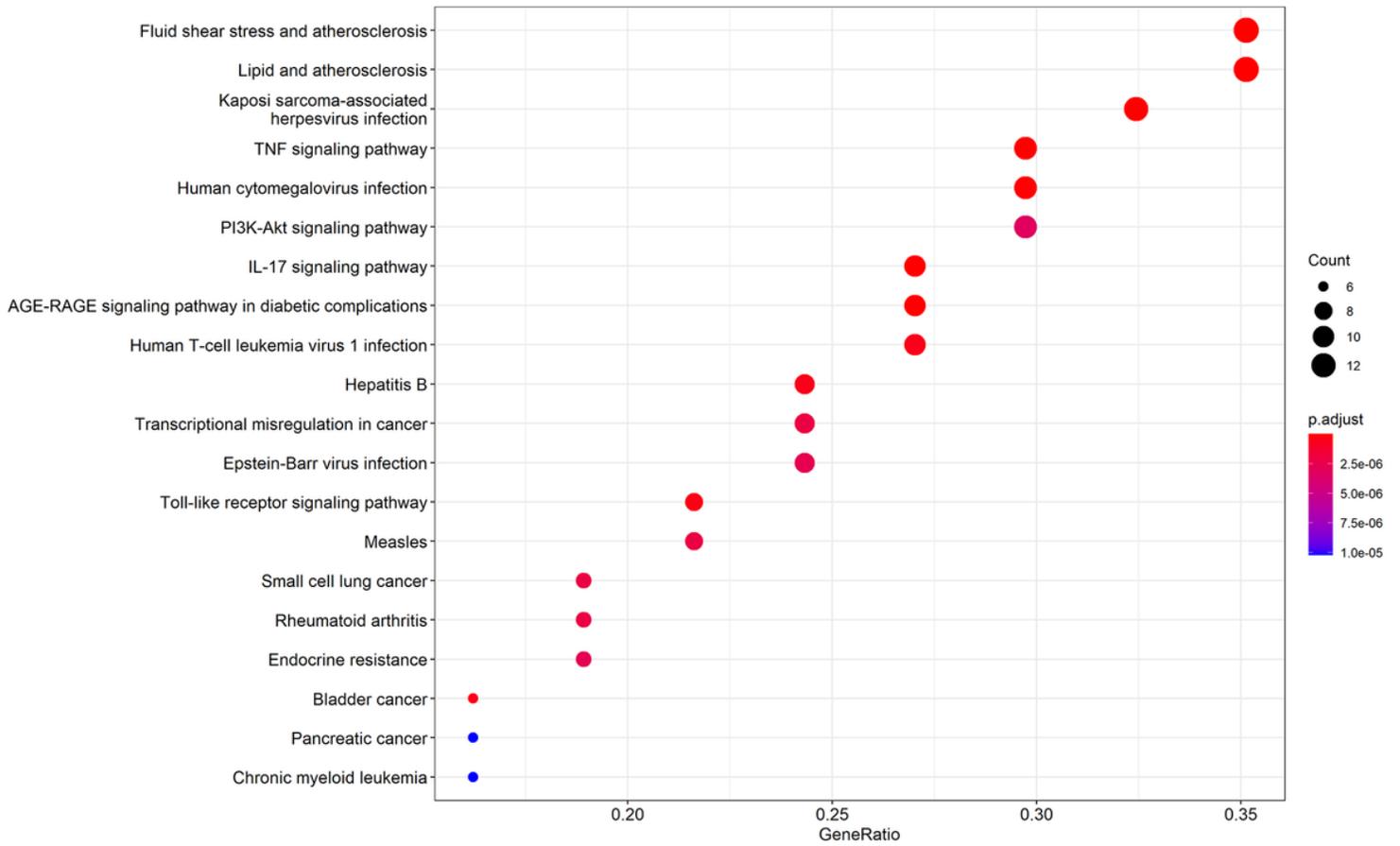


Figure 7

KEGG bubble. The horizontal axis of the KEGG bubble diagram represents the gene proportion enriched in each entry, and the vertical axis shows the enrichment degree according to the corrected P value.

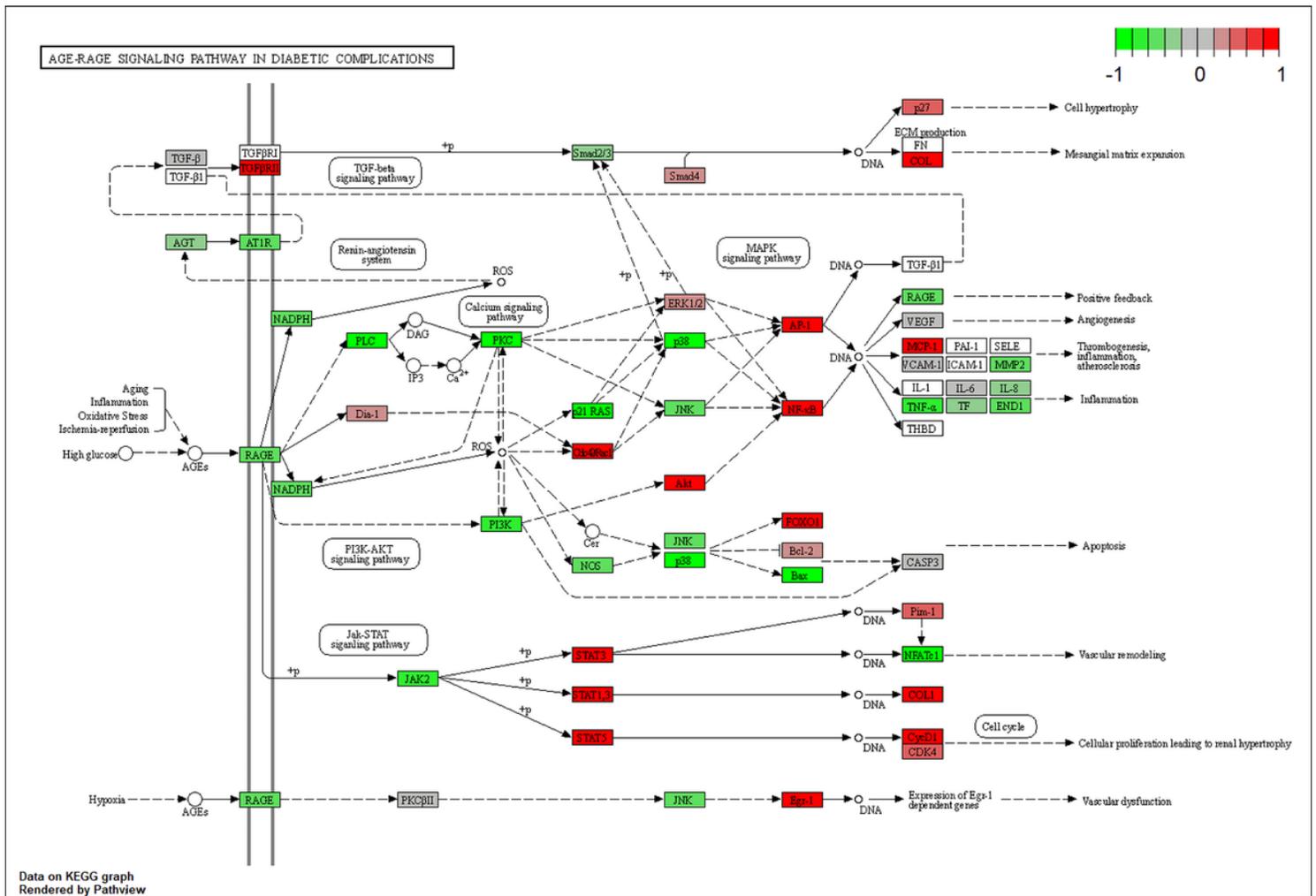


Figure 9

Gene expression in the AGE-RAGE signaling pathway in diabetic complications pathway, the more red color indicates that the target is up-regulated; the more green color indicates that the target is down-regulated.

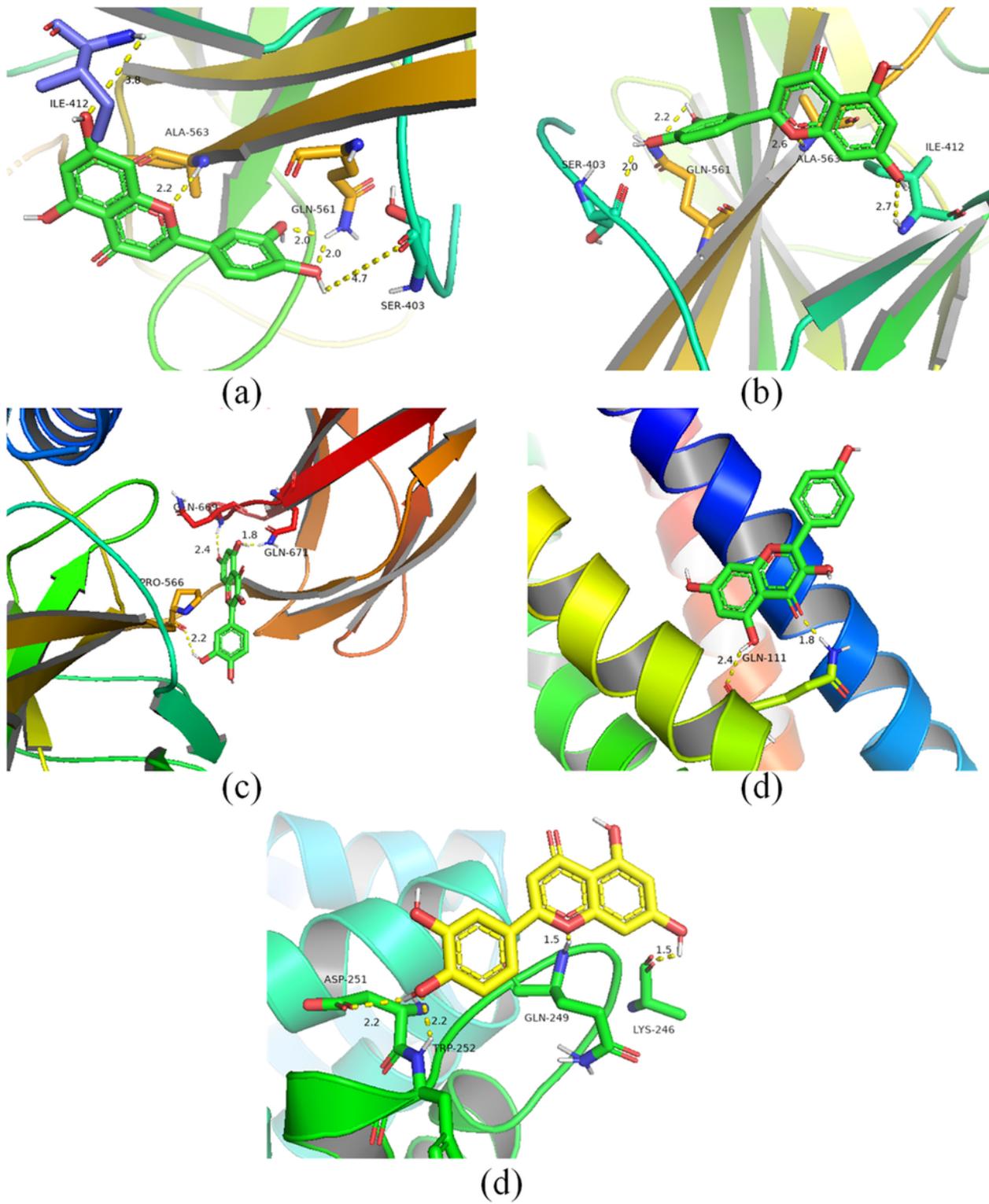


Figure 10

Partial diagram of molecular docking: (a) FOS-kaempferol; (b) FOS-luteolin; (c) FOS-quercetin; (d) IL6-kaempferol; (e)MYC-luteolin.