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A Study on the Mechanism of Milkvetch Root in the treatment of Diabetic Nephropathy based on Network Pharmacology

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Abstract

Background: Diabetic nephropathy (DN) is one of the most common complications of diabetes mellitus. Milkvetch Root has been extensively used to treat DN in clinical practice in China for many years, but the active ingredients, drug targets, and its exact molecular mechanism are not known. The aim of this study was to decrypt the underlying mechanisms of Milkvetch Root in the treatment of DN by using a systems pharmacology approach.

Methods: The components and targets of Milkvetch Root were analyzed using the Traditional Chinese Medicine Systems Pharmacology database. Then we found the common target of Milkvetch Root and disease, constructed a protein-protein interaction (PPI) network using String, and screened the key targets from these common targets through topological analysis. Analyses of enrichment of Gene Ontology (GO) pathways and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Subsequently, the major hubs were imported to the Database for Annotation, Visualization and Integrated Discovery to perform a pathway enrichment analysis.

Results: There were 20 active compounds of Milkvetch Root and 10 diabetic nephropathy -associated targets (AKT1, VEGFA, IL6, PPARG, CCL2, NOS3, SERPINE1, CRP, ICAM1, SLC2A4) that were obtained. Then, the results of GO and KEGG pathway enrichment analyses suggested that the AGE-RAGE signaling pathway in diabetic complications, HIF-1 signaling pathway, PI3K-Akt signaling pathway and TNF signaling pathway in diabetic complications might serve as the key points and principal pathways for DN treatment.

Conclusions: In brief, Milkvetch Root has multiple components, multiple targets and multiple pharmacological effects in the treatment of DN, which provides clues for further research on DN.

1. Background

According to an epidemiological survey, Chinese adults over the age of 18 who was suffered diabetes mellitus was 10.4% in 2013 ranking first in the world [1]. Diabetic nephropathy (DN) is one of the most common complications of diabetes mellitus [2], and patients with diabetes mellitus have an increased risk of end-stage renal disease (ESRD) [3]. This is because glomerular damage and proteinuria associated with diabetes mellitus (DM) cause tubulointerstitial damage, which eventually leads to ESRD [4]. The incidence rate of DN is also increasing year by year. DN is the single most common cause of end-stage renal disease in many parts of the world including Europe, Japan and the United States, with diabetic patients accounting for 25 to 45% of all patients enrolled in end-stage renal disease programs [5]. The early onset of DN is insidious and difficult to be detected in time, and it is usually difficult to reverse at the end of clinical development. At present, the treatment of diabetic nephropathy mainly includes strict control of blood glucose, blood pressure and antidiabetic drugs, which can only delay the progress of renal damage, but there is no new direct treatment of DN [6]. Moreover, studies have shown that inhibitors of the Renin-Angiotensin-Aldosterone System (RAAS) have significant side effects including

hyperkalemia, which limits their use in a significant proportion of patients with DN [7]. Finally, dialysis or kidney transplantation is needed, which seriously affects the quality of life of patients. Therefore, early intervention treatment of DN is of great significance. Accordingly, the search for complementary and alternative medicine has become a top priority.

Traditional Chinese medicine (TCM) has been used to treat various diseases for thousands of years. TCM also has its unique advantages in the treatment of diabetes and its complications, and is widely used in clinical practice in China [8]. Its advantage lies in the aspects of multiple drugs, synergistic effect, reducing toxic and side effects, improving adaptability and so on [9].

Milkvetch Root (Huang Qi in Chinese), also named as radix astragali, is a TCM from Mongolian milkvetch or membranaceus milkvetch. The effect of Milkvetch Root is to replenish qi and firm the surface, diuresis and support toxin, discharge pus, collect sore and generate muscle [10]. In TCM, it is often used as antiperspirant, diuretic and supplement in the treatment of various diseases, such as abscess, nephritis, diabetes, hypertension, cirrhosis, leukemia and uterine cancer [11.12]. Nowadays, Compound Huanglian Capsule, Danggui Shaoyao San and Liuwei Dihuang Pill have been widely used in the treatment of DN [13.14.15]. Milkvetch Root can be also efficacious for DN treatment, but the active ingredients, drug targets, and the exact molecular mechanism are not known.

Network pharmacology, which is based on the interaction network of diseases, genes, target proteins, and drugs, is a systematic analytical method [16]. In recent years, network pharmacology has been used widely in TCM research [17]. For example, the network pharmacology approach was used to define the active components and potential targets in Mulberry leaf for the treatment of diabetes18]. It can reveal the action mechanism of the drug through the combination of computational biology, systems biology, and “omics” related to target drug [19]. It has also transformed the concept of drug discovery from "one target, one drug" to "network target, multi-component therapy".

In summary, we used network pharmacology to analyze the active ingredients, drug targets, and key pathways of Milkvetch Root in DN treatment. This study aims to further clarify the mechanism of Milkvetch Root treatment of DN to provide ideas and theoretical basis. The workflow of the network-pharmacology approach in the present study is illustrated in **Fig. 1**.

2. Methods

2.1 Data preparation

Taking Milkvetch Root as the research object, all ingredients related to Milkvetch Root were screened by the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; http://lsp.nwu.edu.cn/tcmsp.php). Meanwhile, five important pharmacology-related properties were also obtained from TCMSP, including oral bioavailability (OB), intestinal epithelial permeability (Caco-2 cells), drug-likeness (DL), blood-brain barrier (BBB), drug half-life (HL) and Lipinski's rule (LR), provided for the screening and evaluation of compounds. TCMSP database contains 500 kinds of Chinese herbal

medicines and 30069 kinds of ingredients registered in Chinese Pharmacopoeia (2010 Edition) [20]. 87 herbal ingredients about Milkvetch Root were recorded in this process.

2.2 Screening of Active Ingredients

The key parameters of screening were the Oral bioavailability (OB) and Drug likeness (DL) in the database, and then to screening of the active components of Milkvetch Root. OB is an important indicator for objective evaluation of the internal quality of drugs. The higher the OB of an ingredient, the higher is the likelihood that it can be used clinically [21]. Molecules with OB ≥ 30% were considered to have good OB in the present study. Drug likeness (DL) is a qualitative concept used in drug design, which helps to optimize pharmacokinetics and drug properties, such as solubility and chemical stability. Database dependent DL evaluation approach based on Tanimoto coefficient was applied and shown as $T(a,b)=(a \times b)/(|a|^2+|b|^2-a \times b)$ [22]. In this equation, “a” represents the molecular descriptors of herbal compounds, and “b” displays the average molecular properties of all compounds in DrugBank. Those ingredients with DL ≥ 0.18 were selected. In this study, the active component of Milkvetch Root was the compound satisfying OB ≥ 30% and DL ≥ 0.18.

2.3 Target of active Ingredients of Milkvetch Root

The target of active components of Milkvetch Root was queried by TCMSP target. We removed redundant information. Then, the target was transformed using the UniProt knowledge database (UniProt; https://www.uniprot.org/) with the selected species as Homo sapiens. In the end, we can get the right genetic symbols.

2.4 Acquisition of Gene Targets for DN

We collected gene targets for DN from four sources. The first source was the GeneCards v4.14. (www.genecards.org/,2020.03.20) The correlation score (correlation score ≥ 30) was used as the screening condition, and the screening results were used as the candidate target genes of the disease. The second source was the DrugBank v 4.3 (http://www.drugbank.ca/ ,2020.03.20). The third source was the Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org/,2020.03.20).The last source was the PharmGkb (https://www.pharmgkb.org/ ,2020.03.20) [23]. The key word " diabetic nephropathy "or " DN" was input to obtain the genes related to diabetic nephropathy. It should be noted that we need to remove the duplicates of search elements in these four databases.

2.5 Network construction

We intersected the obtained drug targets with the genes associated with a disease, and then represent it with Venn diagram. Subsequently, we built a network of complex information. The Compound-Target Network was built by connecting the candidate compounds and corresponding targets. In the Target-Disease Network, diseases were connected with the associated candidate targets. The D-I-G-D network was built based on interactions between the drug (Milkvetch Root), ingredients, gene symbols, and disease (DN).

We selected three parameters to evaluate the topological features of every node in the interaction network. Degree reflects the number of connections between network nodes and other nodes. Betweenness is the ratio of the number of shortest paths through that point to the total number of shortest paths in the network. Closeness is through the transmission distance between the node to node importance measure. Degree, Betweenness and Closeness are the main topological parameters to measure the importance of a node in the network, and determine whether a target protein important basis for the key targets [24]. The networks were constructed using Cytoscape v3.7.2 (http://www.cytoscape.org/) [25].

2.6 Construction of a Protein–Protein Interaction (PPI) Network

To explain the interaction between target proteins, the genes of the relevant components in the Milkvetch Root were uploaded to STRING (http://string-db.org,v11)to obtain information on PPI. We searched these gene symbols using the "multiple proteins" option while simultaneously setting the organism to Homo sapiens. we selected medium confidence data > 0.4. The obtained protein interaction data were submitted to Cytoscape3.7.2 to build a PPI network.

2.7 Enrichment of Gene Ontology (GO)Pathway and the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway

In order to further study the related effects of Milkvetch Root in the treatment of diabetic nephropathy, Go biological process enrichment analysis was used to interpret the biological process of diabetic nephropathy of Milkvetch Root target, and then KEGG metabolic pathway enrichment analysis was carried out to study the main metabolic pathway of Astragalus in the treatment of diabetic nephropathy. We used Bioconductor (R)v3.6.2 (http://bioconductor.org/)for analyses.

3. Results

3.1 Screening of Active Ingredients

The active components of Milkvetch Root were retrieved from the TCMSP database, and a total of 87 related components were obtained. There were 20 related components satisfying OB ≥ 30% and DL ≥ 0.18. In summary, 20 ingredients were selected as active ingredients in Milkvetch Root (Table 1).

Table 1

A total of 20 ingredients were selected as the details of the active ingredients of Milkvetch Root in this study.

NO	Mol ID	CAS NO	Components	OB(%)	DL
1	MOL000211	472-15-1	Mairin	55.38	0.78
2	MOL000239	3301-49-3	Jaranol	50.83	0.29
3	MOL000296	465-99-6 474-58-8	hederagenin	36.91	0.75
4	MOL000033	64997-52-0	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
5	MOL000354	480-19-3	isorhamnetin	49.6	0.31
6	MOL000371	15689655	3,9-di-O-methylnissolin	53.74	0.48
7	MOL000374	N/A	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69
8	MOL000378	N/A	7-O-methylisomucronulatol	74.69	0.3
9	MOL000379	94367-42-7	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74	0.92
10	MOL000380	73340-41-7 94367-42-7	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
11	MOL000387	73536-69-3	Bifendate	31.1	0.67
12	MOL000392	485-72-3	formononetin	69.67	0.21
13	MOL000398	N/A	isoflavanone	109.99	0.3
14	MOL000417	20575-57-9	Calycosin	47.75	0.24
15	MOL000422	520-18-3	kaempferol	41.88	0.24
16	MOL000433	33609-88-0 59-30-3	FA	68.96	0.71
17	MOL000438	64474-51-7	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26
18	MOL000439	N/A	isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62
19	MOL000442	N/A	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48

NO	Mol ID	CAS NO	Components	OB(%)	DL
20	MOL000098	73123-10-1 74893-81-5 117-39-5	quercetin	46.43	0.28

3.2 Target prediction and analysis

Twenty target components were obtained from Milkvetch Root, and 393 potential targets of Milkvetch Root were obtained from these 20 components. All the targets related to DN were searched in four databases: GeneCards database, DrugBank database, OMIM database and PharmGkb database. We used Cytoscape3.7.2 software to make the active ingredients and targets of Milkvetch Root into C-T network. At the same time, we also used Cytoscape3.7.2 software to analyze the relationship between the targets of Milkvetch Root and DN, and constructed T-D network. **(Fig. 2 and Fig. 3)** In order to further study the mechanism of Milkvetch Root in the treatment of diabetic nephropathy, we also constructed D-I-G-D network with Cytoscape3.7.2 software. (Fig. 4**)** the green node represents Milkvetch Root and the red node represents DN. Meanwhile, the 6 violet nodes represent the active ingredients in Milkvetch Root; the16 blue nodes represent the overlapping gene symbols between the disease and drug. The edges denote that nodes can interact with each other. The network shows drug may indirectly regulate disease-related proteins, while Milkvetch Root can directly affect these proteins. Figure 5 shows that there are 16 overlaps between 393 disease gene symbols and 180 drug gene symbols. In other words, 16 genetic symbols may be the key to Milkvetch Root 's treatment of DN.

3.3 Analyses of a PPI Network

We constructed a PPI network consisting of 16 nodes and 71 edges **(**Fig. 6a**)**. In this network, nodes represent the target protein and each edge represents the Protein-Protein interaction. The average node degree in this PPI network is 8.88, the degree of each node represents the number of targets that are connected to the target. Figure 6b is a PPI network constructed by submitting the obtained protein interaction data to Cytoscape 3.7.2. In this network, the node size and color are used to reflect the number of combined targets (degree).

We took thefirst 10 proteins in the PPI network, **(Fig. 7)** which includesRAC-alpha serine/threonine-protein kinase(AKT1),Vascular endothelial growth factor A(VEGFA),Interleukin-6(IL6),Peroxisome proliferator activated receptor gamma(PPARG),C-C motif chemokine 2(CCL2),Nitric oxide synthase, endothelial(NOS3),Plasminogen activator inhibitor 1(SERPINE1),C-reactive protein(CRP),Intercellular adhesion molecule 1(ICAM1)and Solute carrier family 2, facilitated glucose transporter member 4(SLC2A4).As seen from **Fig. 7**,AKT1 cloud be related to the other 14 proteins, VEGFA cloud be related to the other 13 proteins, IL6 and PPARG cloud be related to the other 12 proteins. CCL2, NOS3 and

SERPINE1 cloud be related to the other 11 proteins. CRP, ICAM1 and SLC2A4 cloud be related to the other 10 proteins. These results suggested thatthese ten proteins would be the focus of our research of PPIs.

3.4 Analyses of Enrichment of GO Pathways

Analyses of enrichment of the GO pathway were carried out using Bioconductor (R). ($p < 0.01$) **(**Fig. 8a,8b,8c**)** In the graph, the vertical axis represents the GO term. The horizontal axis represents the number of genes in the term. The redder the color, the smaller the value of P. adjust (FDR); it also indicates higher credibility and greater importance. The 16 overlapping gene symbols were mapped to 977 pathways after enrichment of the GOBP pathway. We intercepted the first 20 terms from small to large according to the p-value. The results indicated that numerous biologic processes were involved in DN treatment, including cellular response to peptide(GO:1901653), reproductive structure development(GO:0048608), reproductive system development(GO:0061458), response to insulin(GO:0032868), regulation of leukocyte migration(GO:0002685), cellular response to lipopolysaccharide(GO:0071222), female gonad development(GO:0008585), cellular response to molecule of bacterial origin(GO:0071219), cellular response to insulin stimulus(GO:0032869), development of primary female sexual characteristics(GO:0046545). Meanwhile, there are 14 pathways after enrichment of the GOCC pathway, such as extracellular space, cytosol, extracellular area, etc. And there are also 11pathways after enrichment of the GOMF pathway, such as enzyme binding, protein binding, similar protein binding, etc.

3.5 Analyses of Enrichment of the KEGG Pathway

Analyses of enrichment of the KEGG pathway were also carried out using Bioconductor (R). ($p < 0.01$) **(**Fig. 9**)** In the graph, the vertical axis represents the KEGG pathway. The horizontal axis represents the number of genes in the term. For a brief demonstration, we intercepted the first 20 terms from small to large according to the p-value. The first 10 items are as follows: AGE-RAGE signaling pathway in diabetic complications(hsa04933), HIF-1 signaling pathway(hsa04066), Fluid shear stress and atherosclerosis(hsa05418), Insulin resistance(hsa04931), PI3K-Akt signaling pathway(hsa04151), Influenza A(hsa05164), EGFR tyrosine kinase inhibitor resistance(hsa01521),

4. Discussion

Network pharmacology is a rapidly emerging discipline. It has also transformed the concept of drug discovery from "one target, one drug" to "network target, multi-component therapy"[26]. Because of the advantage of network pharmacology research strategy, it can open up a new and innovative way for the development of traditional Chinese medicine. The purpose of this study is to analyze the active components, target and related signal pathway of Milkvetch Root in improving glycolipid metabolism of diabetic nephropathy by network pharmacology, and to explore the possible mechanism of action.

Using network pharmacological analysis, we found that there are 20 active components and predicted 180 potential targets in Milkvetch Root. The results of T-D network analysis show that there are 360 edges in the network, which represent the interaction between active component and target. Among them,

quercetin has the most potential targets with a total of 136, followed by kaempferol with 51 potential targets. Others, such as 7-O-methylisomucronulatol, formononetin, isorhamnetin and(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c] chromen-3-ol also have more corresponding targets, which are 33, 28, 25, and 19 respectively. The corresponding targets of active ingredients are AKT1, VEGFA, IL6, PPARG, NOS3, etc.

Quercetin is a potent antioxidant flavonoid found in many common medicinal herbs, and possesses a wide spectrum of biologic activities [27]. It also has anti-oxidant, hypoglycemic, hypolipidemic, tumor suppression, and anti-inflammatory effects [28.29.30]. One study showed that quercetin liposome or free quercetin can prevent weight loss, decrease kidney hypertrophy index, decrease blood glucose level and 24-hour urine protein in diabetic nephropathy model rat [31]. Kaempferol is a kind of natural peroxisome proliferator-activated receptor-γ(PPARγ) agonist, and PPARγ agonist has also become a common drug in the treatment of diabetes and its complications [32]. Kaempferol has similar hypoglycemic effect with Rosiglitazone, but its adverse reactions are significantly lower than the latter. It can improve the glucose uptake of 3T3-L1 cells, control blood glucose, and improve the oxidative stress damage of kidney caused by glucose metabolism disorder [33]. There are also studies that suggest that kaempferol can work as RhoA/Rho Kinase inhibitor and may attenuate progression of diabetic complications with specific emphasis on DN [34]. Formononetin is a polyphenolic compound. Formononetin is a potential molecule which increases the expression of SIRT1 in kidney tissue of diabetic, which is an effective molecule to control nephropathy in type 2 diabetes mellitus [35].7-O-methylisomucronulatol has the similar pharmacological effect with formononetin. It can prevent and treat DN by inhibiting the proliferation of mesangial cells and the production of nitric oxide [36].

Isorhamnetin can inhibit the NF-κB signaling activity, decreased the production of inflammatory mediators and attenuated oxidative stress in diabetic rats and glomerular mesangial cells(GMCs), thus reducing urinary albumin filtration, reducing renal damage, improving renal pathological changes and so on [37]. This fully shows the complex network relationship between drugs and targets, and verifies that Milkvetch Root plays the role of improving DN in a multi-component and multi-target way.

In addition, quercetin, kaempferol, formononetin and isorhamnetin are all flavonoids. Studies on the mechanism of action suggested that flavonoids can improve the metabolism of sugar and lipid, enhance insulin resistance, inhibit the activity of relevant glucose metabolic enzymes, and avoid oxidative damage of DM [38.39]. Therefore, it is point out that these components may be the main components of Milkvetch Root. It can be seen that flavonoids of TCM may be a novel drug for diabetic nephropathy, which has a broad prospect of development.

In terms of target, there are three isoforms of AkT: AKT1 (PKBα), AKT2 (PKBβ), and AKT3 (PKBγ), which each have their own physiologic functions [40]. The protein kinase AkT, also known as protein kinase B (PKB), has been shown to regulate a variety of cell functions, and is particularly important for glucose metabolism, cell growth, and cell survival. Therefore, changes in its expression or activity are thought to be involved in the pathogenesis of diabetes and DN [41].

In humans, there are five secreted glycoproteins that make up the VEGF family member: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF) [42]. Previous studies have demonstrated that angiotensin type 1 receptor blocker (ARB) can inhibit the synthesis of VEGF mediated by Ang-II and can effectively treat diabetic nephropathy [43]. VEGF-A is an important regulator of angiogenesis and vascular permeability with a possible pathogenic role in diabetic nephropathy [44.45]. VEGF-A is essential for the normal growth of podocytes. When the expression of VEGF-A was lower than the normal level, the podocytes were damaged [46]. In conclusion, blockade of VEGF-A can effectively restore renal function in diabetic nephropathy.

IL-6 in the pathogenesis of DN and its association with insulin resistance. Studies suggested that IL-6 affect the dynamics of ECM surrounding cells and may increase GBM and endothelial permeability [47]. Current evidence suggests that IL-6 responses are mediated via gp130-STAT3 dependent mechanisms which, on one hand, trigger globally the transition from innate to adaptive immune response, and on the other hand act locally for tissue remodeling and immune cell infiltration [48]. Therefore, the regulation of IL6 target is of great significance in the treatment of DN.

PPARG is a kind of transcription factor activated by ligands. At present, there are three subtypes: PPARα, PPARβ, PPARγ [49]. Some studies have found PPARG is the risk of progression of diabetic nephropathy in China [50].

Nitric oxide (NO) is closely related to kidney by regulating renal hemodynamics, renin secretion, inhibiting renal tubular sodium reabsorption, renal tubular glomerular feedback (TGF) and renal sympathetic nerve activity [51.52]. The synthesis of NO in vivo is closely related to nitric oxide synthetase (NOS2/3). The active components act on NOS related targets, enhance the biological activity of NOS, restore no pathway can down regulate the expression of inflammatory factors, thereby reducing creatinine level, protein filtration rate, and play the role of renal protection [53].

In this study, according to the result of molecular docking and network analysis, all protein-pathway pairs were distributed among oxidative stress, inflammation, metabolism, immune system, apoptosis, and multiple pathways. For instance, Oxidative stress and inflammation prompted by hyperglycemia are the key initiators finally leading to renal damage and nephropathy [54.55]. AGE-RAGE signaling pathway in diabetic complications, HIF-1 signaling pathway, PI3K-Akt signaling pathway and TNF signaling pathway are responsible for imparting therapeutic effects on DN, and these pathways have been widely studied. Some studies have confirmed that AGE-RAGE signaling pathway is a signaling mechanism in the pathogenesis of diabetes and its complications [56]. It can aggravate the vascular damage of diabetes through oxidative stress [57], increase the risk of renal function deterioration and cardiovascular events, leading to an increase in all-cause mortality [58]. HIF can activate in the early stage of DN under hypoxia, and stimulate the proliferation and aggregation of inflammatory factors in the injured kidney, which paves the way for renal fibroblast scar [59.60]. Secondly, HIF can be combined with fibrosis-promoting genes such as collagen 1, connective tissue growth factor, and plasminogen activator inhibitor 1, to generate interstitial collagen and reduce degradation of the extracellular matrix (ECM), eventually leading

to renal fibrosis [61]. PI3K-Akt signaling pathway has been indicated as the source of glomerular hypertrophy and ECM accumulation [62]. PI3K can activate its downstream molecule Akt, and Akt further phosphorylates fox OS, GSK-3, Bad, mTOR and other proteins to cause the cascade reaction of signal pathway, which plays a key role in the accumulation of extracellular matrix, mesangial cell proliferation, epithelial mesenchymal transformation and other aspects of diabetic nephropathy[63.64]. TNF-α can stimulate the aggregation and adhesion of inflammatory cells, increase the permeability of small blood vessels, and damage the glomeruli through inflammatory reactions [65].

5. Conclusions

In this study, the mechanism of astragalus in the treatment of diabetic nephropathy was analyzed by a network pharmacological method. Six active ingredients that can directly affect diabetic nephropathy targets and ten potential targets were found. We suggest that the AGE-RAGE signaling pathway in diabetic complications, HIF-1 signaling pathway, PI3K-Akt signaling pathway and TNF signaling pathway in diabetic complications might serve as the key points and principal pathways for DN treatment. To summary, we explored, systematically, how Milkvetch Root may operate in terms of DN treatment. The data mentioned above also suggest that Milkvetch Root has multiple ingredients, multiple targets, and multiple approaches. Such data provide the basis for multi-ingredient synergies in future research.

Abbreviations

DN: Diabetic nephropathy; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; ESRD :end-stage renal disease ; DM: diabetes mellitus ;RAAS: Renin-Angiotensin-Aldosterone System; TCM :Traditional Chinese medicine; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB: oral bioavailability; Caco-2cells :intestinal epithelial permeability; DL: drug-likeness; BBB: blood-brain barrier; HL: drug half-life; LR: Lipinski's rule ; OMIM: Online Mendelian Inheritance in Man; AKT1: RAC-alpha serine/threonine-protein kinase; VEGFA : Vascular endothelial growth factor A; IL6: Interleukin-6; PPARG :Peroxisome proliferator activated receptor gamma; CCL2:C-C motif chemokine 2; NOS3:Nitric oxide synthase, endothelial; SERPINE1: Plasminogen activator inhibitor; CRP : C-reactive protein; ICAM1:Intercellular adhesion molecule 1 ; SLC2A4: Solute carrier family 2, facilitated glucose transporter member 4;GOCC: Gene Ontology cellular component;GOMF: Gene Ontology molecular function; PPARγ: peroxisome proliferator-activated receptor-γ; GMCs: glomerular mesangial cells; PKB: protein kinase B; ARB: angiotensin type 1 receptor blocker; PlGF :placental growth factor; ECM: extracellular matrix; NO: Nitric oxide.

Declarations

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Authors' contributions

F-ML and X-LT conceived and designed the research methods. C-LP, QZ and DJ collected the data. C-LP and DJ analyzed the data. CT, LW and N-WZ provided the technical support. QZ wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

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

Consent for publication

The manuscript is approved by all authors for publication.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

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Figures

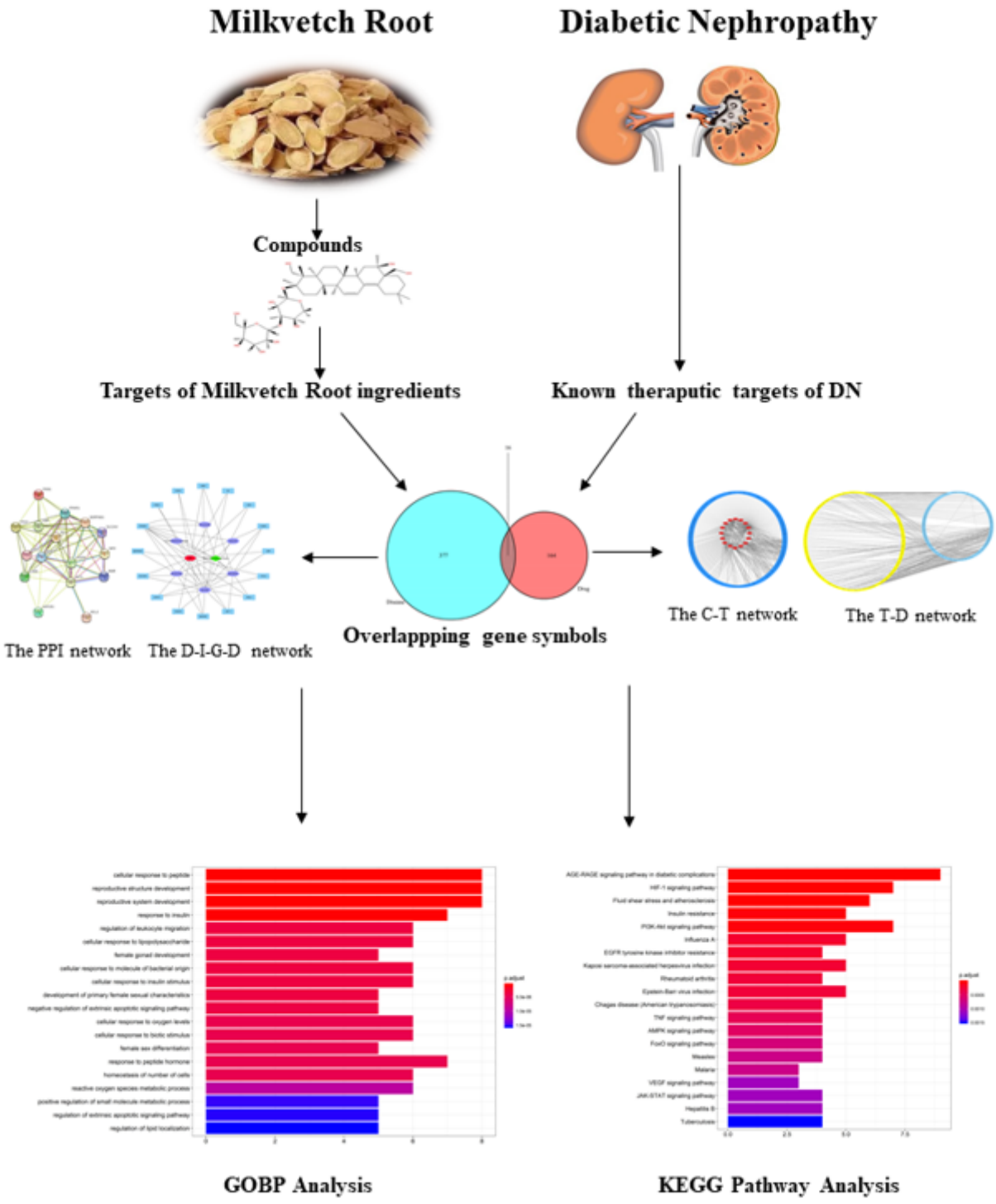

Figure 1

Flowchart of a network pharmacology-based strategy to investigate the pharamacologic mechanisms of Milkvetch Root for treatment of Diabetic Nephropathy.

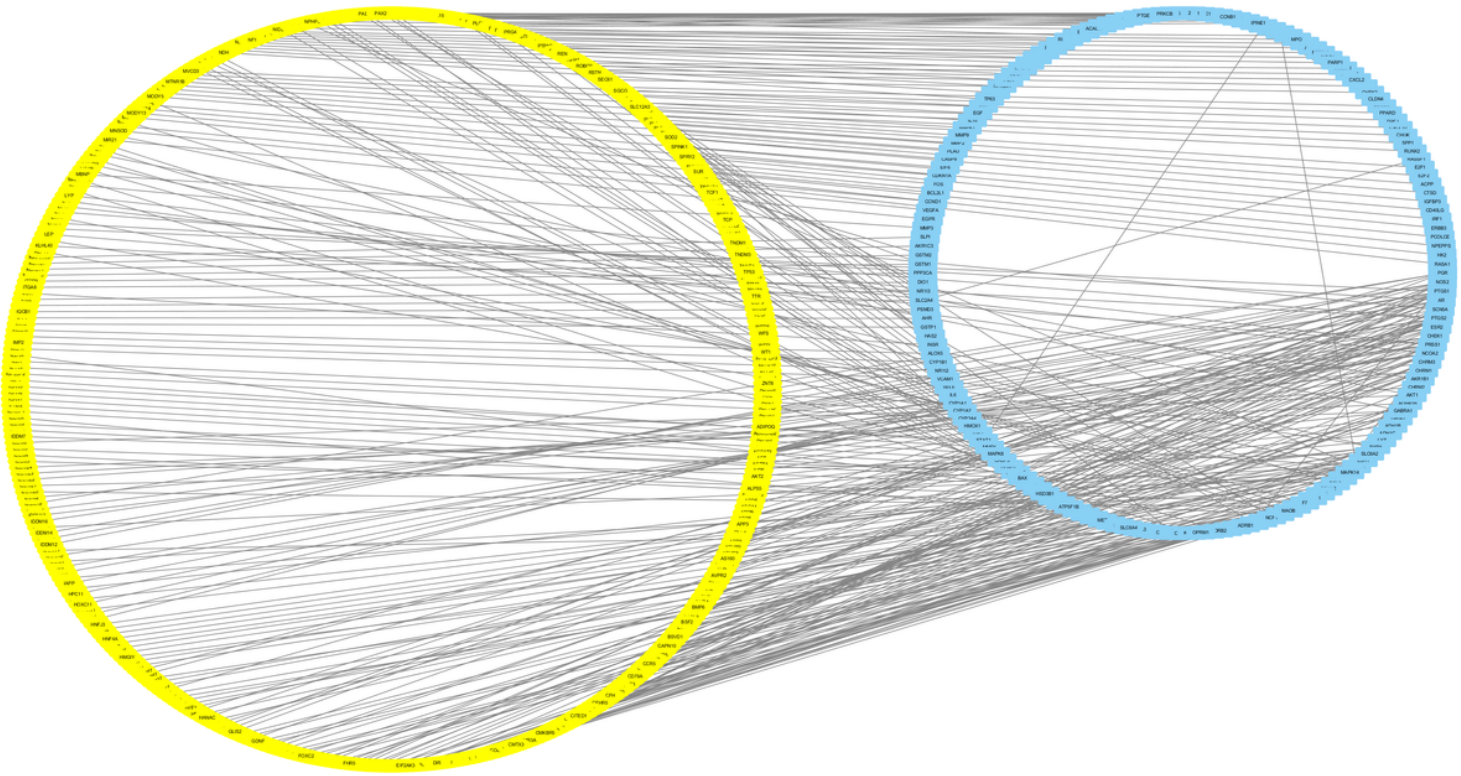

Figure 2

The T-D network that consists of 393 nodes and 360 targets. yellow and blue nodes denote the diseases and targets, respectively.

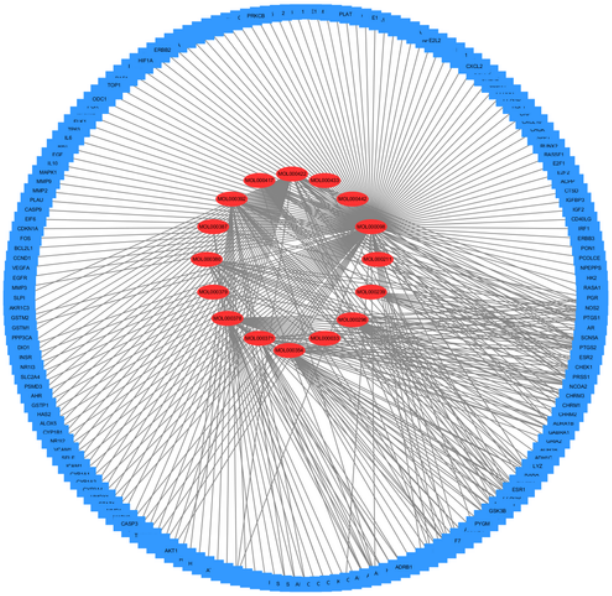

Figure 3

The C-T network that consists of 16 nodes and 360 targets. Red and blue nodes denote the compounds and targets, respectively

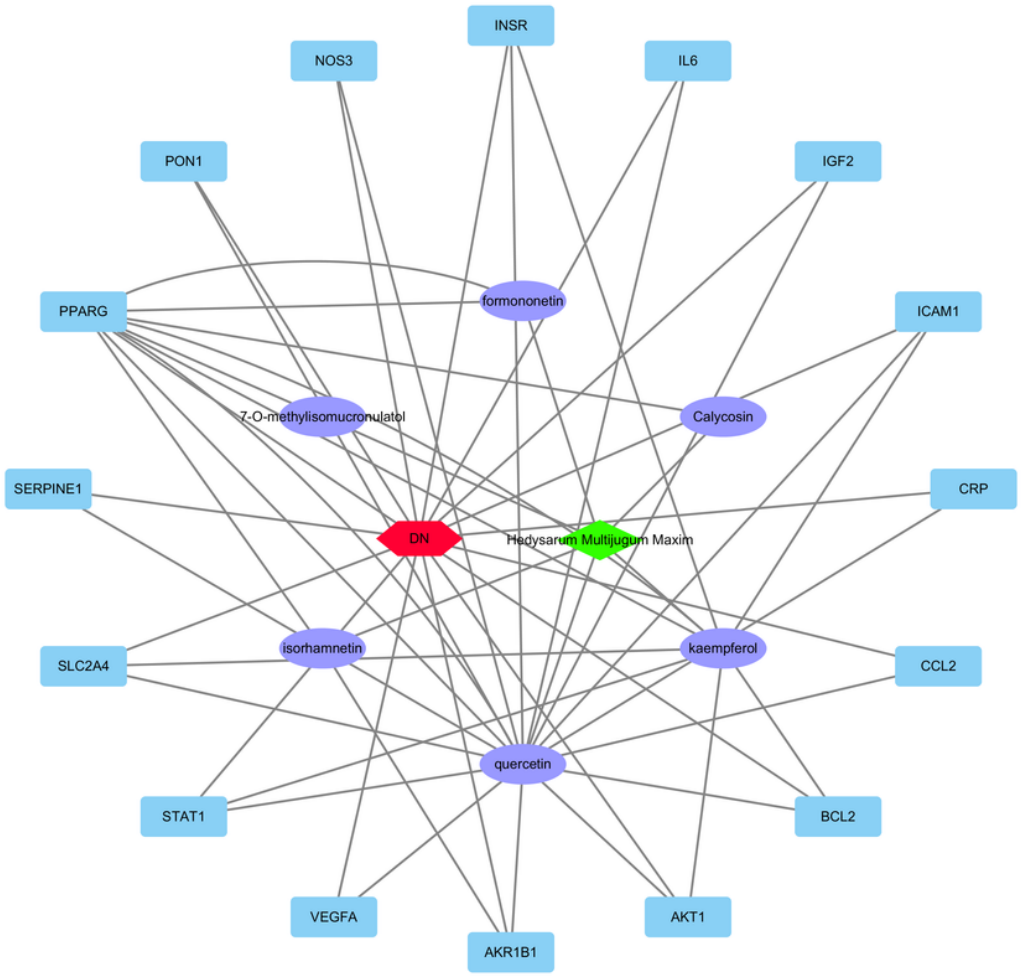

Figure 4

The D-I-G-D network. The green node represents Milkvetch Root and the red node represents DN. The 6 violet nodes represent the active ingredients in Milkvetch Root; The 16 blue nodes represent the overlapping gene symbols between the disease and drug. The edges denote that nodes can interact with each other.

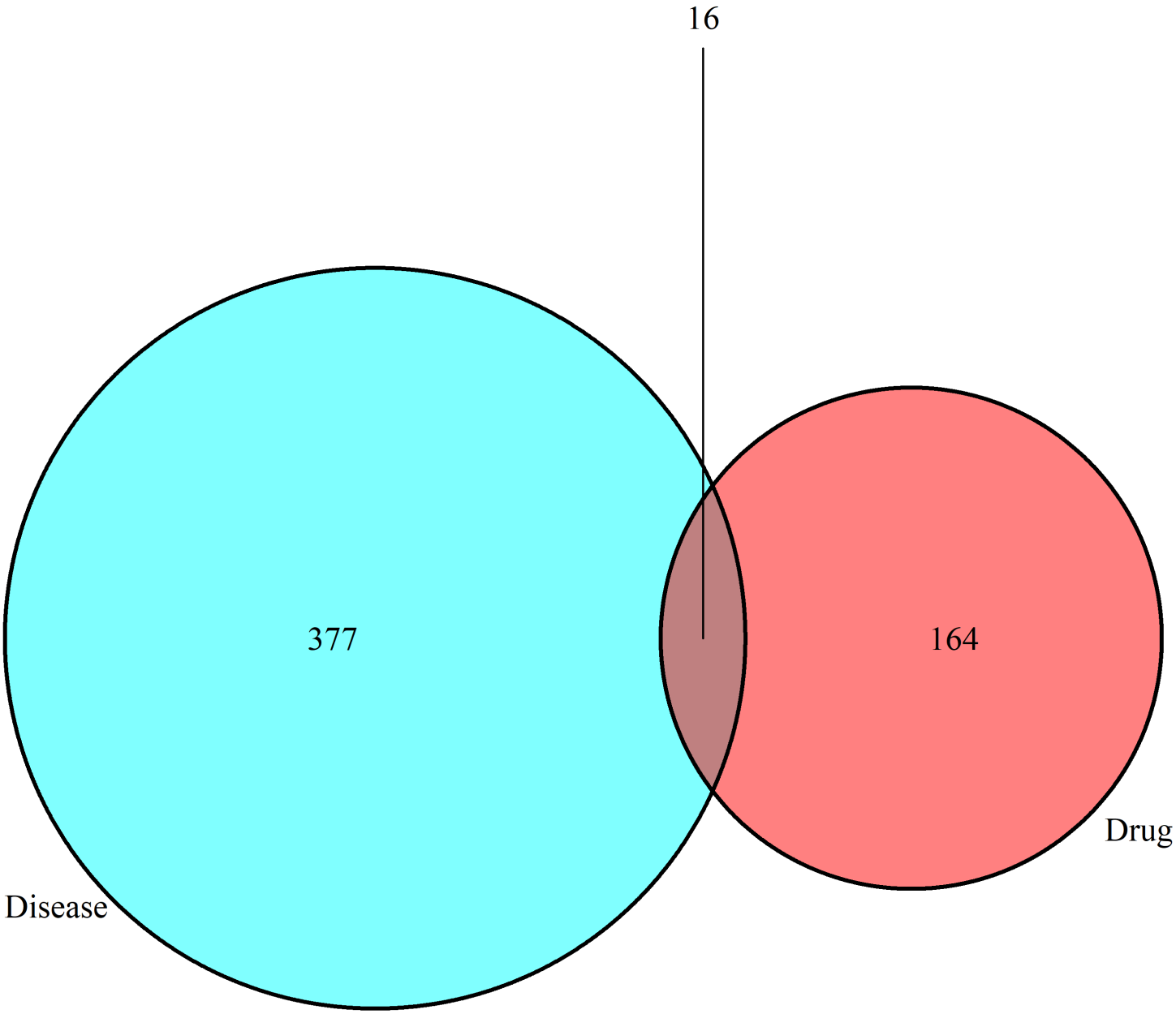

Figure 5

The 16 matching targets of the related targets in Milkvetch Root on DN. DR diabetic retinopathy.

Figure 6

6a PPI network of 16 nodes and 71 edges established in the String Datebase. 6b PPI network of 16 nodes and 71 edges established in the Cytoscape 3.7.2.

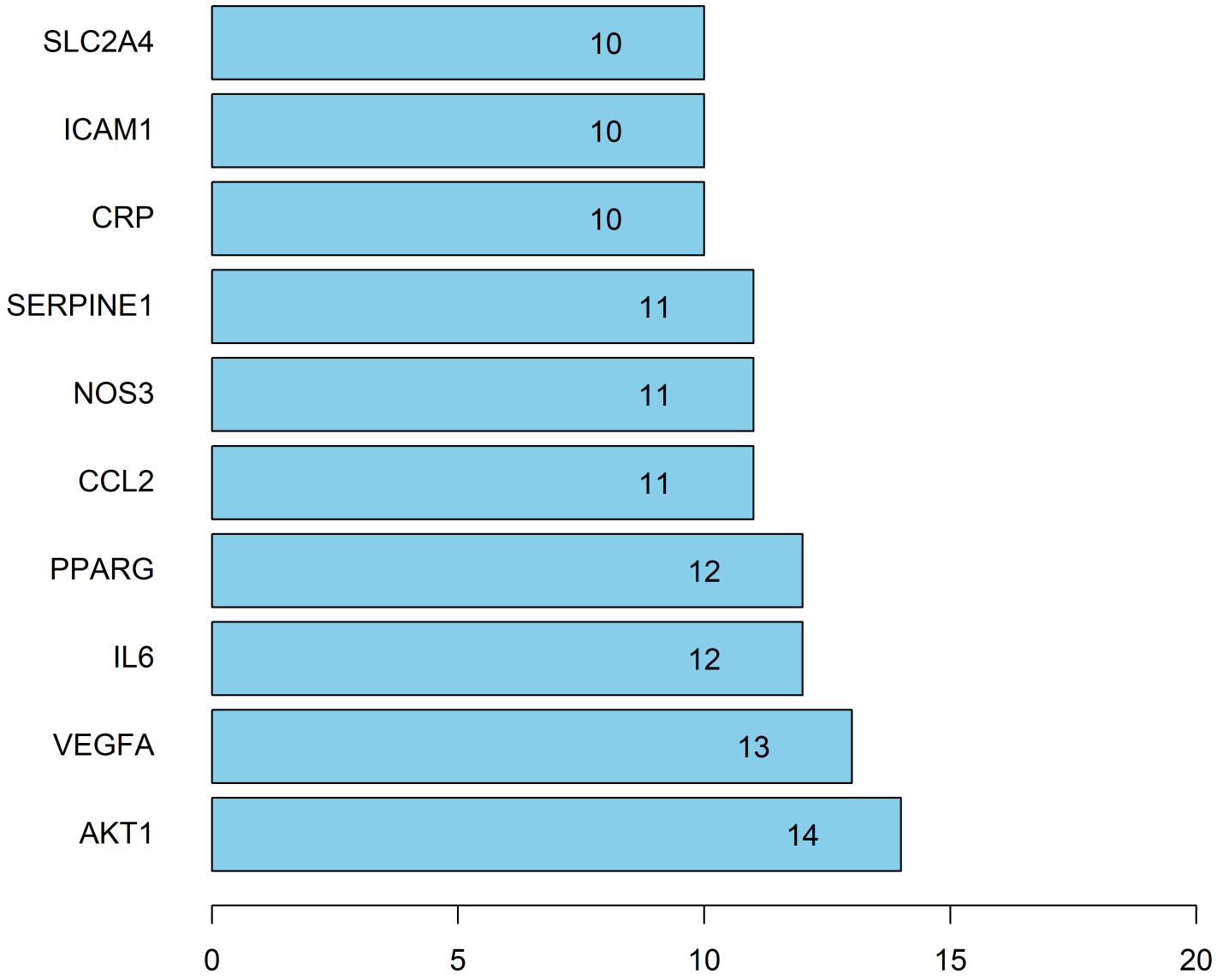

Figure 7

The bar plot of the PPI network. The x-axis represents the number of neighboring proteins of the target protein. The y-axis represents the target protein.

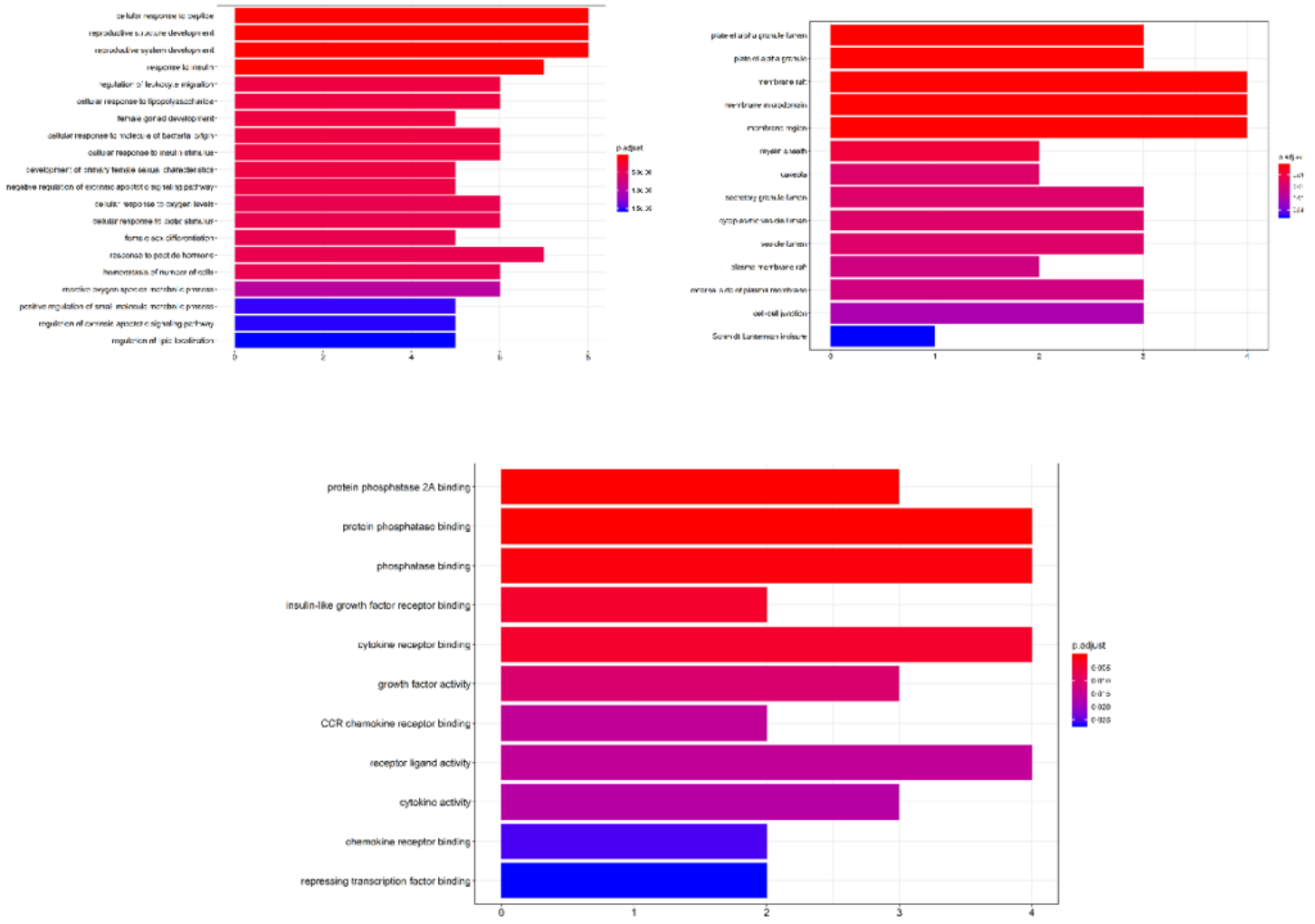

Figure 8

a: Main 20 GO biological process;b: Main 14 GO cellular component;c: Main 11 GO molecular function. (P<0.05)

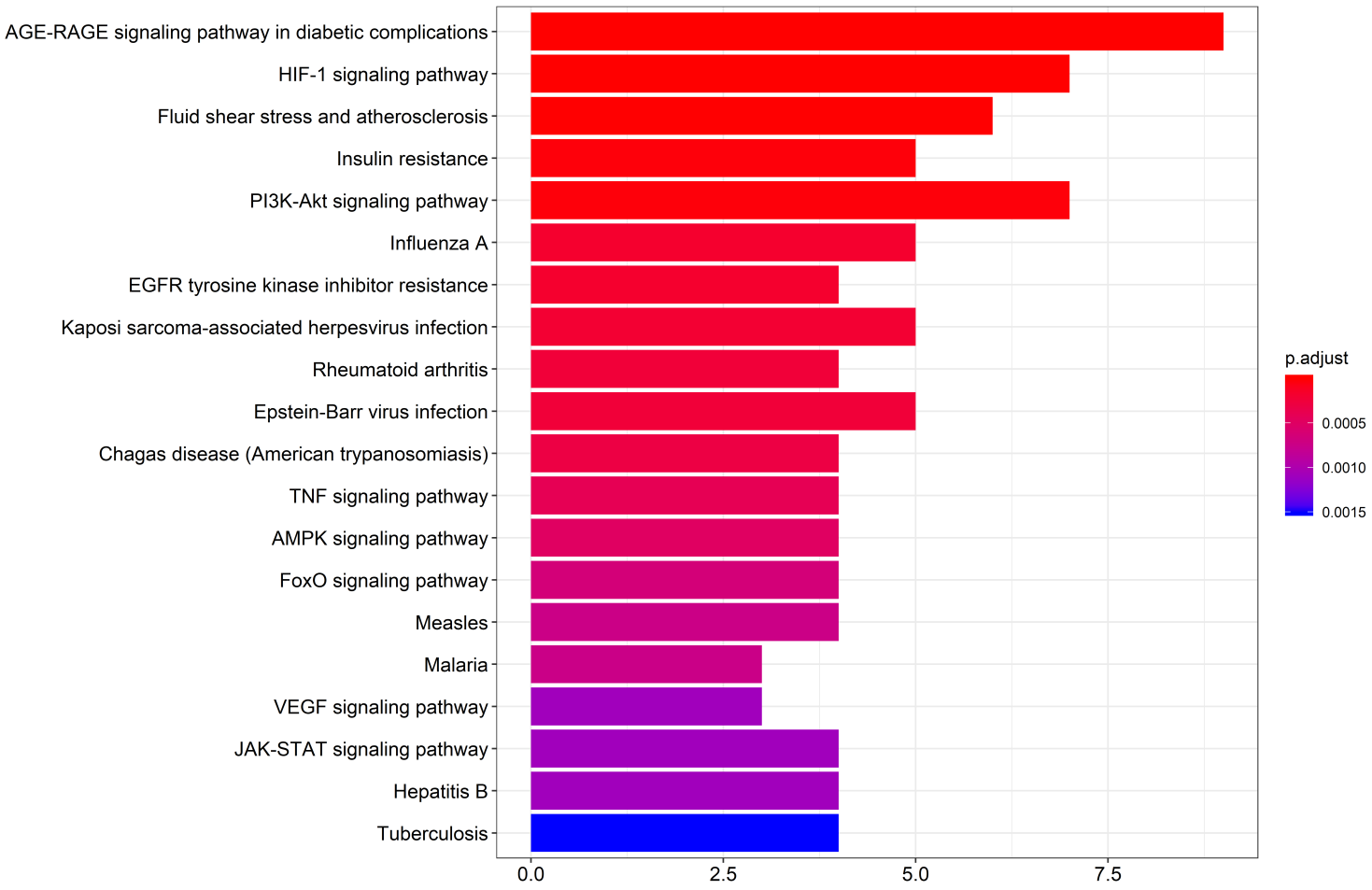

Figure 9

KEGG pathway enrichment analyses. The x-axis represents the counts of the target symbols in each pathway. The y-axis represents the main pathway. (P<0.05)