Research on the Potential Mechanism of Chuanxiong Rhizoma on Treating Diabetic Nephropathy Based on Network Pharmacology

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Research

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Abstract

Background: Chuanxiong Rhizoma is one of the traditional Chinese medicines which have been used for years in the treatment of diabetic nephropathy (DN). However, the mechanism of Chuanxiong Rhizoma in DN has not yet been fully understood.

Methods: We performed network pharmacology to construct targeted proteins interaction network of Chuanxiong Rhizoma. Active ingredients were acquired from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. DRUGBANK database was used to predict targeted proteins of Chuanxiong Rhizoma. Gene ontology (GO) biological process analyses and KEGG pathway enrichment analyses were also performed for functional prediction of the targeted proteins. Molecular docking was applied for evaluating the drug interactions between hub targets and active ingredients.

Results: Twenty-eight targets fished by 6 active ingredients of Chuanxiong Rhizoma were obtained in the study. The top 10 significant GO analyses, as well as 6 KEGG pathways, were enriched for genomic analysis. We also acquired 1366 differentially expressed genes associated with DN from GSE30528 dataset, including five targeted genes: KCNH2, NCOA1, KDR, NR3C2 and ADRB2. Molecular docking analysis successfully combined KCNH2, NCOA1, KDR and ADRB2 to Myricanone with docking scores from 4.61 to 6.28. NR3C2 also displayed good binding forces (8.13 and 8.34) with Wallichilide and Sitosterol, revealing good binding forces to active compounds of Chuanxiong Rhizoma.

Conclusions: Chuanxiong Rhizoma might take part in the treatment of DN through pathways associated with steroid hormone, estrogen, thyroid hormone and IL-17. KCNH2, NCOA1, KDR, ADRB2 and NR3C2 were proved to be the hub targets closely related to corresponding active ingredients of Chuanxiong Rhizoma.

1. Background

Diabetic nephropathy (DN) is a common microvascular complication of diabetes mellitus (DM), which is also the most chronic complication of DM with high risk of disability and difficulty in curing [1]. About 20% ~ 40% of diabetic patients are accompanied with DN [2], who suffer from 6 times of risk of developing into end-stage renal disease (ESRD) [3]. Although many therapies have been tested in animal models, there is still a lack of effective therapeutic drugs for DN since the pathogenesis of DN is very complex, which has not yet been fully understood.

Traditional Chinese medicine (TCM) has been used for years in the treatment of DN [4]. Chuanxiong Rhizoma is one of these TCM which was usually used in the treatment of brain and heart diseases [5] in the past. Isolated from the alkaloid extracted from Chuanxiong Rhizoma, tetramethylpyrazine has multiple pharmacological effects including anti-oxidation, improving microcirculation, and inhibition the production of glycation [6]. Current studies have also revealed that Chuanxiong Rhizoma could inhibit endothelial cell damage [7], as well as vascular smooth muscle cell proliferation [8]. Chuanxiong Rhizoma could also reduce IL-6, IL-8 and TNF-α level in serum to reduce inflammation [9]. What's more, Chuanxiong

is a frequently prescribed component in many TCM formulas for the therapy of DN[10][11]. Some researchers have shown that Chuanxiong injection can provide protective effect in patients with DN[12], improve renal function (blood urea nitrogen [BUN] and serum creatinine [SCr]) and reduce in urine protein[13]. However, the mechanism of Chuanxiong Rhizoma in DN has not yet been fully studied.

Using complex network and visualization technology, network pharmacology can combine with TCM to treat diseases with multiple targets, multiple pathways and multi-target synergy, which can provide new ideas and effective measures for TCM mechanism research. In this study, we performed a network pharmacology research focus on Chuanxiong Rhizoma to further explore the possible mechanism of its action on DN, and provide a solid theoretical basis for the follow-up study.

2. Methods And Materials

2.1 Active ingredients acquiring and targeted protein predicting

Based on Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php) [14] and DRUGBANK database(https://www.drugbank.ca/) [15], we acquired active ingredients of Chuanxiong Rhizoma by according to the suggested drug screening criteria: oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. The DRUGBANK database provides accurate and reliable prediction of the target proteins corresponding to chemical small molecules of active ingredients. Gene symbol associated with target proteins could be acquired from the uniprot database(https://www.uniprot.org/) [16]. The action targets related with DN were also screened from the comparative toxicogenomics database (CTD)(http://ctdbase.org/) [17]. And then common targets for Chuanxiong Rhizoma and DN were obtained using Venn diagram. The flow diagram of this study was shown in Fig. 1.

2.2 Construction of targeted proteins interaction network

The STRING database (https://string-db.org/cgi/input.pl?sessionId=FE22IrLmPAsy&input_page_show_search=on) was used to analyze the targeted proteins interactions. The minimum required interaction score was set at 0.4 for network diagram. And then targeted proteins interaction network was constructed using Cytoscape 3.5.1 software (http://www.cytoscape.org) [18].

2.3 Functional prediction of targeted proteins of Chuanxiong Rhizoma

Based on the interactions of targeted proteins, we performed Gene ontology (GO) biological process analyses and KEGG pathway enrichment analyses to predict the potential function of targeted proteins of

Chuanxiong Rhizoma. The clusterProfiler package in R was used for statistical analysis and visualization of functional profiles for genes and gene clusters [19].

2.4 Hub proteins designation for DN treated with Chuanxiong Rhizoma

Raw gene data and corresponding clinical data of microarray profile from GSE30528 dataset [20] using the Human Genome U133A 2.0 Array were acquired from Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/gds/), and then background adjusted by Robust Multichip Average [21]. GSE30528 dataset includes microarray data of 9 DN glomeruli tissues and 13 controlled normal glomeruli tissues. The limma package [22] available from the Bioconductor project was performed to find out differentially expressed genes (DEGs) between DN glomeruli tissues and controlled normal glomeruli tissues. Absolute value of fold change of gene signature more than 1.5 and *P* value less than 0.05 were set as the significance threshold. The targeted proteins associated with DEGs of diabetic nephropathy would be selected as hub proteins for subsequent docking analysis.

2.5 Molecular docking of hub proteins

In order to evaluate the drug interactions with potential protein targets involved in this research, the selected hub proteins were evaluated by SystemsDock with the high-precision docking simulation assessing protein-ligand interaction [23]. The molecular formulas of active ingredients were obtained from the TCMSP database. Structure files of targeted proteins were acquired from RCSB Protein Data Bank (PDB database, http://www.pdb.org/pdb/home/home.do) [17]. Docking score, ranging from 0 to 10 representing weak combining ability to strong combining ability, represents a negative logarithm of experimental dissociation/inhibition constant value (pKd/pKi) [24].

2.6 Statistical analysis

R 3.6.1 (www.rproject.org) and SPSS 13.0 (SPSS Inc., Chicago, IL, USA) were used for data analysis. *P* value less than 0.05 was considered significant. We also applied Cytoscape 3.5.1 software to visualize the compound-target-pathway interaction network of Chuanxiong Rhizoma in this study.

3. Results

3.1 Active ingredients and targeted protein of Chuanxiong Rhizoma

Seven active ingredients of Chuanxiong Rhizoma (Mandenol, Myricanone, Perlolyrine, Senkyunone, Wallichilide, Sitosterol and FA) were acquired from TCMSP database according to the suggested screening criteria: OB ≥ 30% and DL ≥ 0.18 (Table 1). One active ingredient (Senkyunone) without any corresponding target was excluded. Based on the reliable prediction of the target protein from the DRUGBANK database, we finally obtained 28 targetd proteins of Chuanxiong Rhizoma after excluding the repeated targets (Table 2). Compound-target network of Chuanxiong Rhizoma was shown in Fig. 2.

We searched “diabetic nephropathy” in the CTD database and found 18652 DN-related genes. Ultimately, Venn diagram summarized 28 common targets both associated with DN and Chuanxiong Rhizoma (Fig. 3a) for further analysis. Targeted protein interaction network of Chuanxiong Rhizoma from STRING was shown in Fig. 3b.

Table 1
Characteristics of the seven active ingredients of Chuanxiong Rhizoma

Molecule ID	Molecule name	Molecular weight	OB (%)	DL
MOL000433	FA	441.45	68.96	0.71
MOL002140	Perlolyrine	264.3	65.95	0.27
MOL002151	Senkyunone	326.52	47.66	0.24
MOL002157	Wallichilide	412.57	42.31	0.71
MOL001494	Mandenol	308.56	42	0.19
MOL002135	Myricanone	356.45	40.6	0.51
MOL000359	Sitosterol	414.79	36.91	0.75
OB: oral bioavailability; DL: drug-likeness.				

3.2 GO biological process analyses and KEGG pathway enrichment analyses

Twenty-eight targets fished by 6 active ingredients of Chuanxiong Rhizoma and DN from CTD database were further analyzed for functional prediction. The top 10 significant GO analyses, as well as 6 KEGG pathways, were enriched for genomic analysis. In GO biological processes analysis (Fig. 3c), the targets were mainly enriched in response to steroid hormone (GO:0048545); rhythmic process (GO:0048511); steroid hormone mediated signaling pathway (GO:0043401); cellular

Table 2

Predicted target from active ingredients

Target Name	UniProt ID	Gene code	General Function
Nitric oxide synthase, inducible	P35228	NOS2	Tetrahydrobiopterin binding
Prostaglandin G/H synthase 1	P23219	PTGS1	Prostaglandin-endoperoxide synthase activity
Potassium voltage-gated channel subfamily H member 2	Q12809	KCNH2	Voltage-gated potassium channel activity involved in ventricular cardiac muscle cell action potential repolarization
Estrogen receptor	P03372	ESR1	Zinc ion binding
Androgen receptor	P10275	AR	Zinc ion binding
Sodium channel protein type 5 subunit alpha	Q14524	SCN5A	Voltage-gated sodium channel activity involved in sa node cell action potential
Peroxisome proliferator activated receptor gamma	P37231	PPARG	Zinc ion binding
Prostaglandin G/H synthase 2	P35354	PTGS2	Prostaglandin-endoperoxide synthase activity
Coagulation factor VII	P08709	F7	Serine-type peptidase activity
Vascular endothelial growth factor receptor 2	P35968	KDR	Vascular endothelial growth factor receptor binding
Retinoic acid receptor RXR-alpha	P19793	RXRA	Zinc ion binding
CGMP-inhibited 3',5'-cyclic phosphodiesterase A	Q14432	PDE3A	Metal ion binding
Beta-2 adrenergic receptor	P07550	ADRB2	Protein homodimerization activity
Estrogen receptor beta	O95718	ESRRB	Zinc ion binding
Dipeptidyl peptidase IV	P27487	DPP4	Virus receptor activity
Mitogen-activated protein kinase 14	Q16539	MAPK14	Protein serine/threonine kinase activity
Glycogen synthase kinase-3 beta	P49841	GSK3B	Ubiquitin protein ligase binding
Heat shock protein HSP 90	P07900	HSP90AB1	Tpr domain binding
Serine/threonine-protein kinase Chk1	O14757	CHEK1	Protein serine/threonine kinase activity

Target Name	UniProt ID	Gene code	General Function
Ig gamma-1 chain C region	P01857	IGHG1	Immunoglobulin receptor binding
Proto-oncogene serine/threonine-protein kinase Pim-1	P11309	PIM1	Transcription factor binding
Cyclin-A2	P20248	CCNA2	Essential for the control of the cell cycle at the G1/S (start) and the G2/M (mitosis) transitions
Nuclear receptor coactivator 1	Q15788	NCOA1	Transcription coactivator activity
Nuclear receptor coactivator 2	Q15596	NCOA2	Transcription coactivator activity
Mineralocorticoid receptor	P08235	NR3C2	Zinc ion binding
Glucocorticoid receptor	P04150	NR3C1	Zinc ion binding
Cell division protein kinase 2	P24941	CDK2	Metal ion binding
Progesterone receptor	P06401	PGR	Zinc ion binding

response to drug (GO:0035690); hormone-mediated signaling pathway (GO:0009755); cellular response to steroid hormone stimulus (GO:0071383); reproductive structure development (GO:0048608); intracellular receptor signaling pathway (GO:0030522); transcription initiation from RNA polymerase II promoter (GO:0006367); DNA-templated transcription, initiation (GO:0006352). Figure 3d reveals the enriched KEGG pathways of the targets, including progesterone-mediated oocyte maturation (hsa04914), thyroid hormone signaling pathway (hsa04919), estrogen signaling pathway (hsa04915), IL-17 signaling pathway (hsa04657), small cell lung cancer (hsa05222) and prostate cancer (hsa05215).

3.3 Construction of compound-target protein-pathway network of Chuanxiong Rhizoma

Compound-target protein-pathway network of Chuanxiong Rhizoma was constructed using Cytoscape 3.5.1 software. The interaction network was constituted by 6 active ingredients of Chuanxiong Rhizoma, 28 corresponding targeted protein (shown by gene symbol), 10 gene ontology biological process and 6 KEGG pathways. As shown in Fig. 4, squires represent active compounds of Chuanxiong Rhizoma, rotundities represent gene symbols of targets, hexagons represent enriched GO biological processes and diamonds represent enriched KEGG pathways.

3.4 Hub proteins designation and molecular docking

Based on GSE30528 dataset, 1366 DEGs associated with DN were found according to the significance threshold: absolute value of fold change of gene signature more than 1.5 and *P* value less than 0.05. 1366 DEGs were shown as green plots in Fig. 5a, including five targeted genes: KCNH2, NCOA1, KDR, NR3C2 and ADRB2 (red plots in Fig. 5a). Further analysis revealed that gene signatures of KCNH2, NCOA1, KDR and NR3C2 were significantly lower in DN glomeruli tissues compared to controlled normal glomeruli tissues, while gene signature of ADRB2 was significantly higher in DN glomeruli tissues (Fig. 5b). The corresponding five targeted proteins were designed as hub proteins with ligand-protein interaction diagrams (Fig. 5c) for further molecular docking analysis.

With the help of SystemsDock [23], we successfully combined KCNH2, NCOA1, KDR and ADRB2 to Myricanone with docking scores from 4.61 to 6.28. What more, NR3C2 also displayed good binding forces (8.13 and 8.34) with Wallichilide and Sitosterol (Fig. 5d), revealing good binding forces to active compounds of Chuanxiong Rhizoma.

4. Discussion

As one of the principal causes of ESRD around the world, DN is closely related to cardiovascular and cerebrovascular diseases and increased mortality of DM patients [25]. DN is characterized by proteinuria and changes in renal ultrastructure, and the prognosis of patients with DN is poor, especially for these patients with ESRD. It takes place approximately a quarter of patients with type 2 DM and about 33% of type 1 DM patients [26][27]. Due to the limitations in the understanding of DN, currently, there is still a lack of accurate treatment strategies specifically targeting at DN in addition to controlling blood sugar, blood lipid levels and hypertension.

In this study, we carried out a network pharmacology research focus on Chuanxiong Rhizoma, one of the important TCM used in treating DN, to further explore its possible mechanism on DN. By applying GO biological process analyses, the special targets of Chuanxiong Rhizoma were significantly enriched in hormone-mediated signaling pathway, response to steroid hormone, and other pathways related to reproductive structure development. Previous studies have suggested that steroid hormone might play a vital role in the pathogenesis of DN in DM patients [28][29][30]. Estradiol could inhibit the transcription of type IV collagen and reduce the expression of collagen by way of activating tyrosine kinase 2 and inhibiting the synthesis of TGF-β, so as to alleviate fibrosis in DN patients [31]. Our study revealed that Chuanxiong Rhizoma might target at steroid hormone in the treatment of DN.

KEGG pathway enrichment analyses suggested that Chuanxiong Rhizoma was associated with estrogen signaling pathway, thyroid hormone signaling pathway and IL-17 signaling pathway in the treatment of DN. Estrogen has been reported to act on renal protection by up-regulating the level of endothelial NO synthase and inhibiting the synthesis of inducible NO synthase to maintain normal renal function [32] [33]. DN patients are often accompanied by hypothyroidism, and even low triiodothyronine syndrome. Serum free triiodothyronine acts as a key predictor of prognosis in patients with DN [34]. In addition, thyroid hormone replacement therapy could reduce the risk of cardiovascular diseases in DN patients

with hypothyroidism [35]. Recently, the relationship between IL-17 and DN has attracted more and more attentions from the researchers. Patients with DN are reported to have elevated levels of IL-17 in their peripheral blood [36]. Animal study showed that activation of Thl7 and increase of IL-17 expression play important roles in the pathogenesis of DN [37]. Here, with the help of network pharmacology analysis, our study revealed that Chuanxiong Rhizoma might take part in the treatment of DN through pathways associated with estrogen, thyroid hormone and IL-17.

GSE30528 dataset [19] contains microarray data of 9 DN glomeruli tissues and 13 controlled normal glomeruli tissues. By setting the significance threshold of absolute value of fold change of gene signature > 1.5 and *P* value < 0.05, 1366 DEGs associated with DN were obtained, including five hub genes/proteins targeted by Chuanxiong Rhizoma. In our study, KCNH2, NCOA1, KDR, ADRB2 and NR3C2 are closely related to corresponding active ingredients of Chuanxiong Rhizoma with favorable molecular docking scores, indicating good binding relationships between compounds and targeted proteins. Among the hub targets, KCNH2 is involved in ventricular cardiac muscle cell action potential repolarization, while NCOA1 is enriched in thyroid hormone signaling pathway and estrogen signaling pathway. ADRB2 regulates beta-2 adrenergic receptor (β2AR), which has been reported to act on inhibiting of macrophage function [38] and be involved in LPS-induced activation of THP-1 cells [39]. Agonists of β2AR are involved in the regulation of macrophage activation in diabetic cardiovascular and renal complications [40].

There are still some shortcomings in our study. Firstly, we just explored the potential functional mechanism of Chuanxiong Rhizoma on DN, without analyzing the mutual interferences of drug components. Secondly, this study failed to perform stratification analysis of different pathological stages of DN. Finally, the key targets and pathways we obtained here have not been experimentally verified. Further molecular mechanism researches and clinical validations will be carried out in our future work.

5. Conclusions

Chuanxiong Rhizoma has been proven to play a role in the treatment of DN. Through network pharmacology analysis, our study revealed that Chuanxiong Rhizoma might take part in the treatment of DN through pathways associated with steroid hormone, estrogen, thyroid hormone and IL-17. Molecular docking supported the application of KCNH2, NCOA1, KDR, ADRB2 and NR3C2, which were closely related to corresponding active ingredients of Chuanxiong Rhizoma. However, further molecular mechanism researches and clinical validations are still needed in the future.

Abbreviations

DN: diabetic nephropathy; GO: Gene Ontology; DM; diabetes mellitus; ESRD: end-stage renal disease; TCM: Traditional Chinese Medicine; BUN: blood urea nitrogen; SCr: serum creatinine; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB: oral bioavailability; DL: drug-likeness; CTD: comparative toxicogenomics database; KEGG: Kyoto Encyclopedia of Genes and Genomes; DEGs: differentially expressed genes; PDB: Protein Data Bank.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript is approved by all authors for publication.

Availability of data and materials

The data and materials generated or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

H-SS and C-ST conceived and designed the research methods. L-ZL and W-YH collected the data. C-ST analyzed the data. H-SS wrote the paper. H-SS and C-ST contributed equally to this work. W-Y supervised this research. All authors read and approved the final manuscript.

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Figures

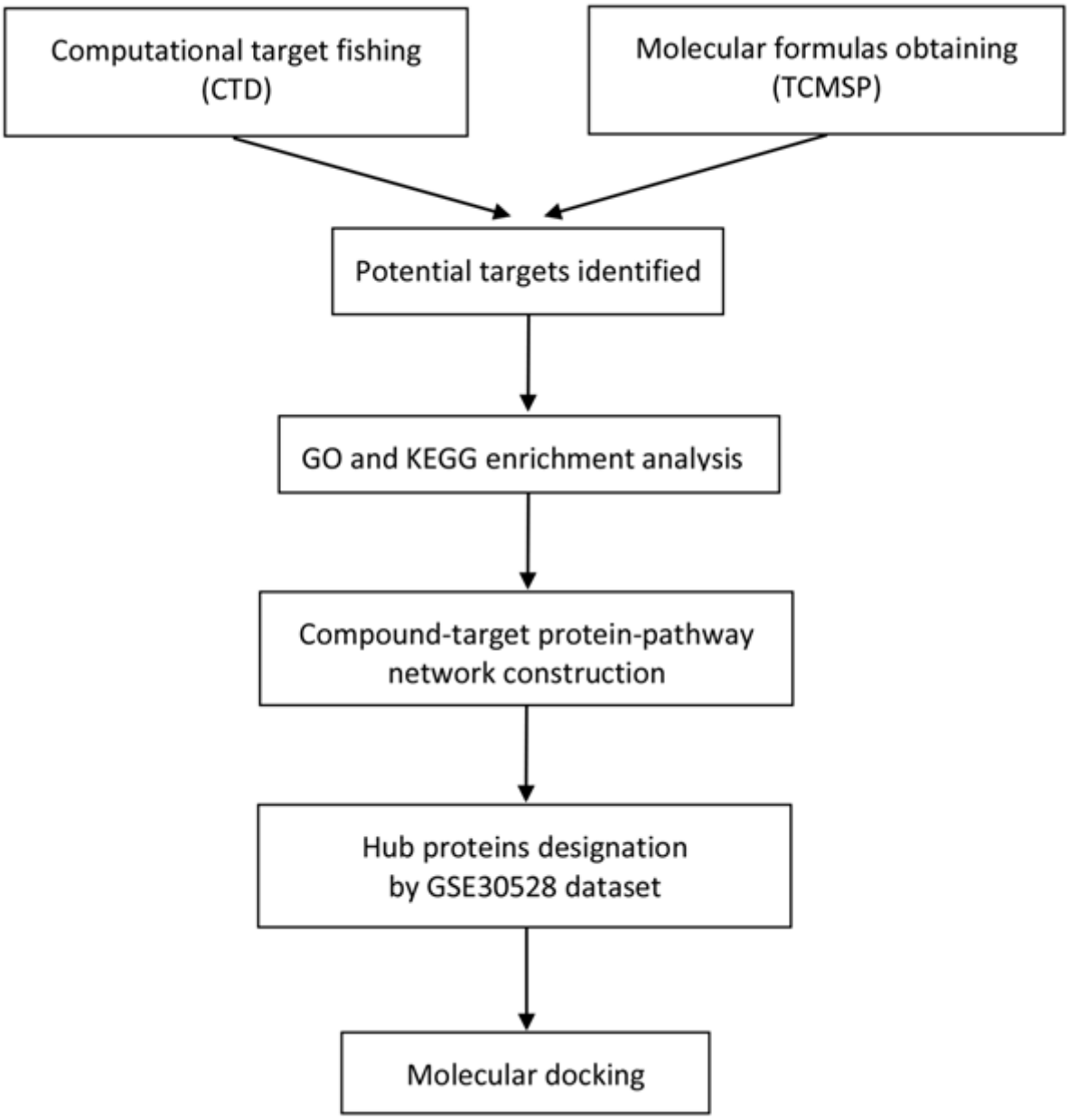

Figure 1

Flow diagram of the research

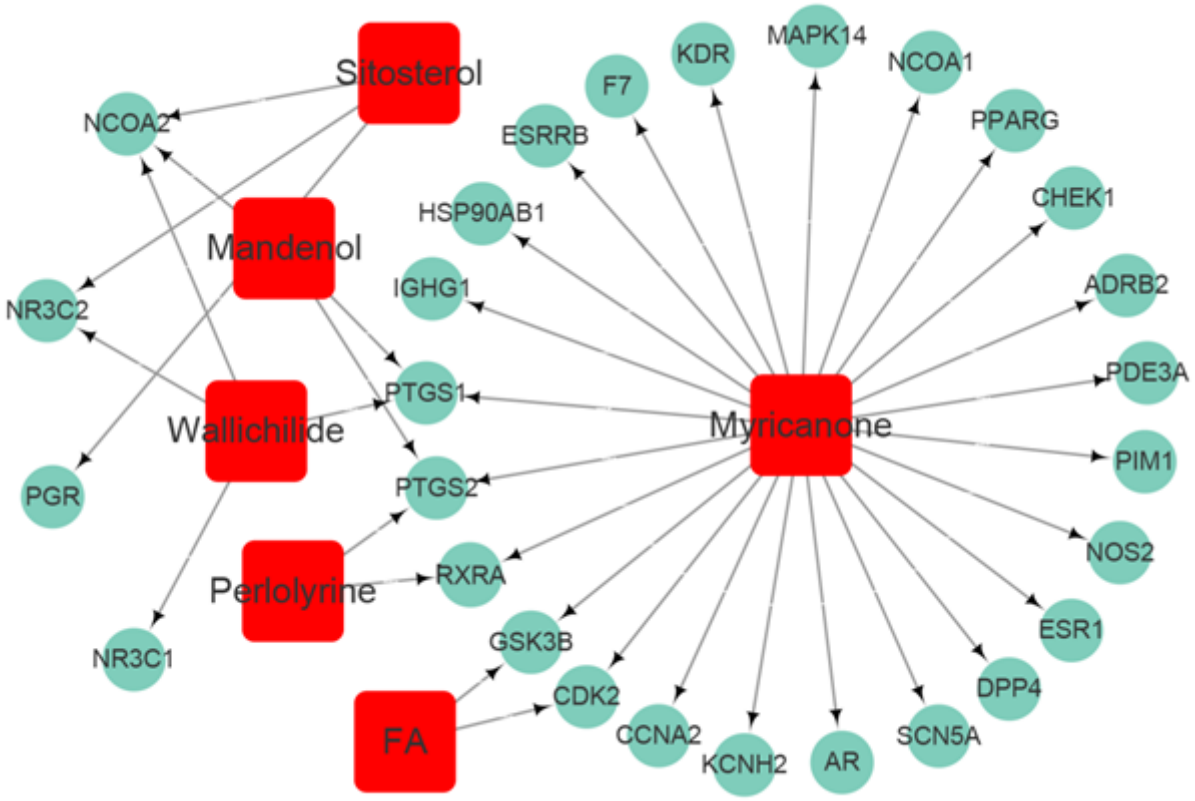

Figure 2

Compound-target network of Chuanxiong Rhizoma. Squires represent active compounds of Chuanxiong Rhizoma. Rotundities represent gene symbols of targets.

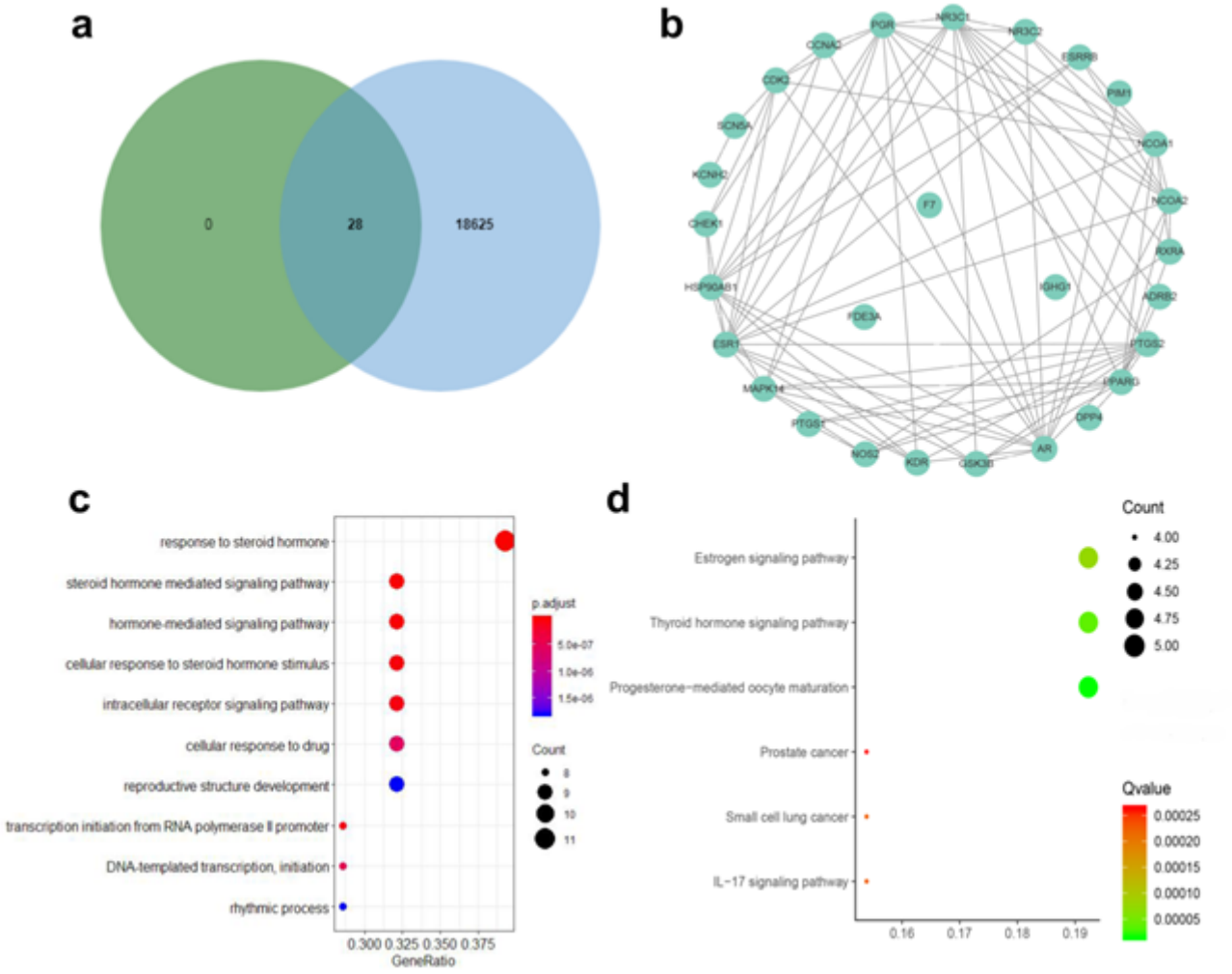

Figure 3

Venn diagram summaries common targets associated with diabetic nephropathy and Chuanxiong Rhizoma; b. Targeted protein interaction network of Chuanxiong Rhizoma from STRING; c. Gene ontology enrichment analysis of 28 specialized targets; d. KEGG pathway enrichment analysis of 28 specialized targets.

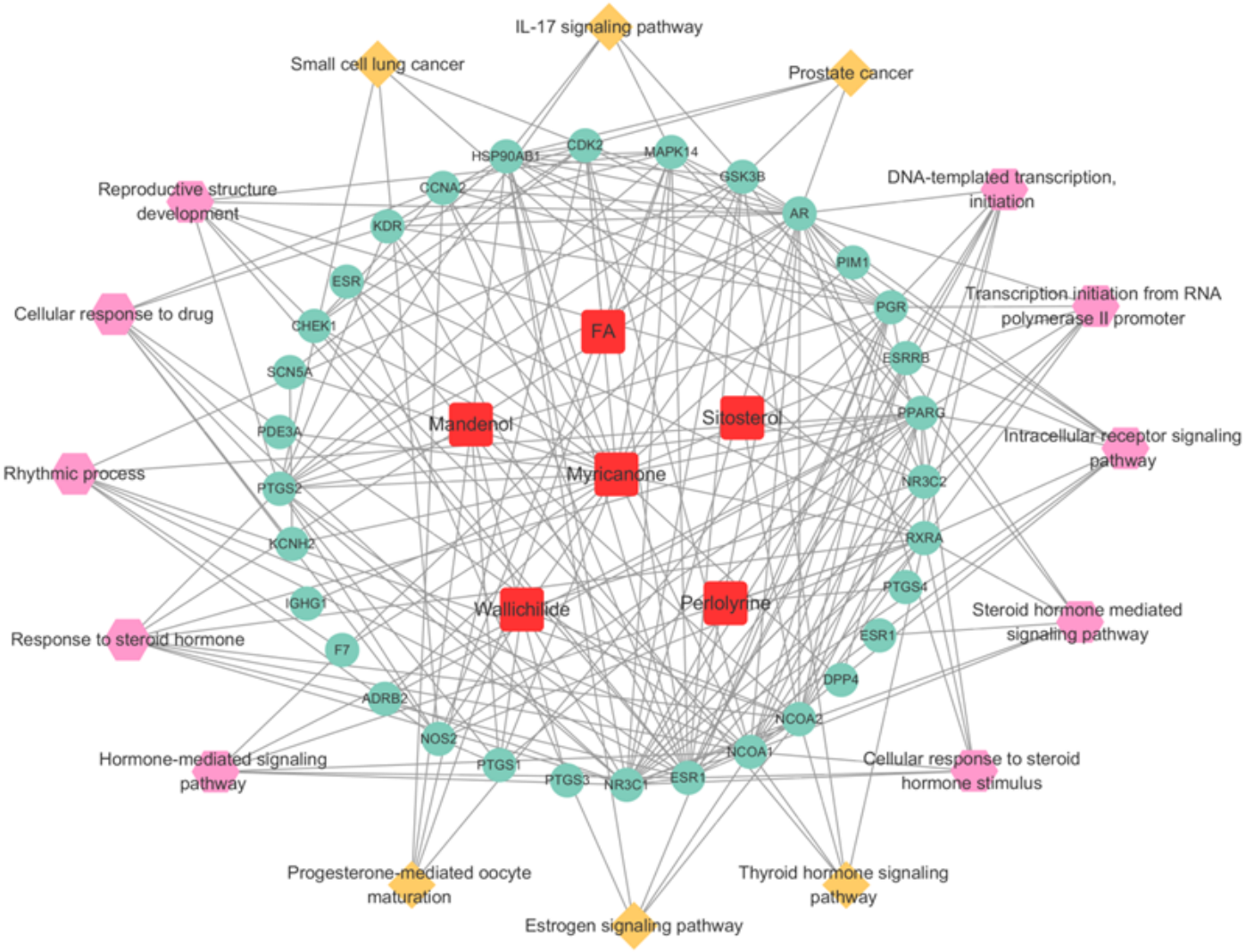

Figure 4

Prediction of compound-target protein-pathway network of Chuanxiong Rhizoma. Squires represent active compounds of Chuanxiong Rhizoma. Rotundities represent gene symbols of targets. Hexagons represent enriched gene ontology biological processes. Diamonds represent enriched KEGG pathways.

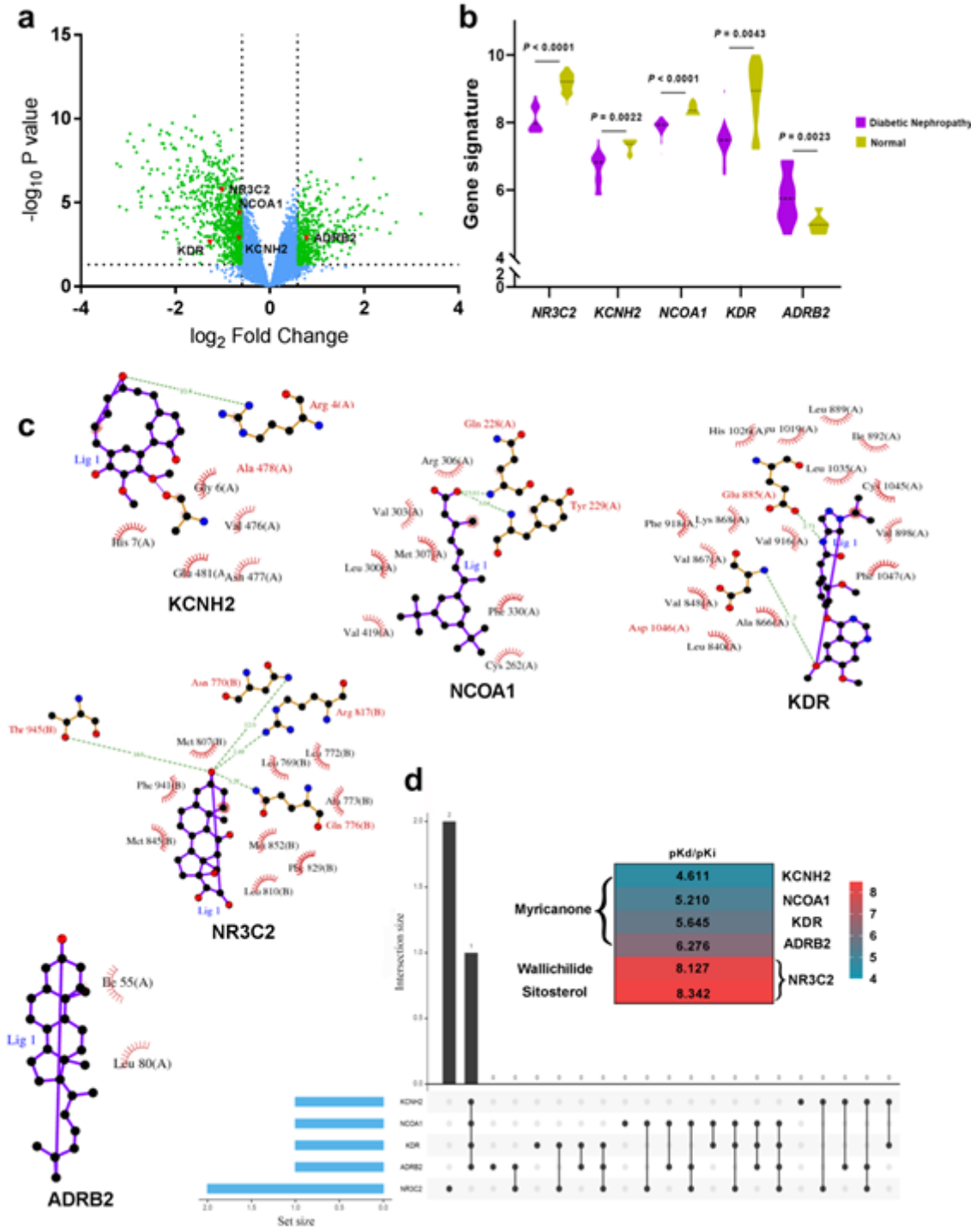

Figure 5

Volcano Plot represents differentially expressed genes (DEGs) associated with diabetic nephropathy from GSE 30528 dataset and 5 hub proteins (gene symbol: KCNH2, NCOA1, KDR, NR3C2 and ADRB2) targeted by Chuanxiong Rhizoma; green plots represent DEGs; red plots represent hub genes/proteins; b. Different gene signatures of hub genes in diabetic nephropathy patients compared with controlled patients in GSE 30528 dataset; c. Ligand-protein interaction diagrams of hub proteins; d. Heatmap shows docking scores (pKd/pKi) of hub proteins combining to active compounds of Chuanxiong Rhizoma.