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Research paper

Network pharmacology—Deciphering the molecular mechanism of San-Zi-Yang-Qin decoction for the treatment of chronic obstructive pulmonary disease

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ABSTRACT

Introduction: San-Zi-Yang-Qin Decoction (SZYQD) is a common traditional Chinese herbal medicine, which has been used for centuries in the treatment of Chronic Obstructive Pulmonary Diseases (COPD). In order to investigate the molecular mechanism of SZYQD in the treatment of COPD, we analyzed the effective active ingredients, potential targets, cooperative functions and associated pathways based on network pharmacology.
Methods: Active ingredients were collected and identified to predict their targets from TCMSP database. Predicted targets related to COPD were screened with CTD to establish a Drugs-components-targets network and Proteins interactions network using STRING Database and Cytoscape software. Action targets were highlighted by pictures of correlative pathways using KEGG PATHWAY Database.
Results: 29 effectively active ingredients were filtered out, including Stigmasterol, beta-carotene, beta-sitosterol, luteolin, linolenic acid, METHYL LINOLEATE, etc. There were 170 targets predicted and all of them were related to COPD, including Heme oxygenase 1 (HMOX1), Glutathione S-transferase P 1 (GSTP1), Phosphatidylinositol 3-kinase (PI3K), Apoptosis regulator Bcl-2 (Bcl-2), Extracellular regulated protein kinases (ERK), Transcription factor p65 (RELA), NF-kappa-B inhibitor alpha (NFKBIA), Matrix metalloprotease (MMP), Cellular tumor antigen p53 (TP53), Caspase, Muscarinic acetylcholine receptor M1-5 (CHRM1-5), etc, enriched with a number of relevant GO functions and KEGG pathways.
Conclusion: SZYQD may regulate the Cholinergic synapse, Th17 cell differentiation, Fluid shear stress and atherosclerosis, Apoptosis, PI3K-Akt signaling pathway, NF-κB signaling pathway, TNF signaling pathway, C-type lectin receptor signaling pathway and so on, to resist oxidation, diminish inflammation, balance proliferation and apoptosis of cells, inhibit vagal excitation in the treatment of COPD.

1. Introduction

1.1. Epidemiology of chronic obstructive pulmonary disease

Chronic Obstructive Pulmonary Disease (COPD) is a lung disease characterized by sustained airflow restriction. Airflow restriction is not completely reversible and develops progressively, mainly involving the lungs, and can also cause damage to extrapulmonary organs. Smoking is the main cause of COPD, followed by environmental pollution, infectious factors, and climate, etc. Reports suggest that the worldwide prevalence of COPD in adults over the age of 40 ranges from 5% to 19% and it is estimated that COPD will rise from the fifth leading cause of death worldwide in 2001 to the third by 2020 [1]. However, pharmacological therapy for COPD mainly focuses on reducing symptoms, the frequency severity of exacerbations and so on. Treatment includes bronchodilators, β2-agonists, antimuscarinic drugs, methylxanthines, etc, which are well-known to be associated with various adverse reaction [2]. For example, β2-agonist, such as Salbutamol and Formoterol, can be the cause of muscle tremors and heart palpitations; Antimuscarinic drugs, such as Ipratropium Bromide and Anisodamine, can cause dry mouth and uroschesis; Methylxanthines may lead to asitia, emesis, agrypnia and even arrhythmia, psychiatric disorders, gastrorrhagia, coma, etc; Glucocorticoid, like Beclomethasone and Fluticasone, may lead to pharyngeal fungal infection. Therefore, it's necessary to explore additional treatment in order to improve symptoms and provide a better treatment experience to patients within a shorter time.

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1.2. Pharmacology of San-Zi-Yang-Qin decoction

Traditional Chinese medicine (TCM) prescriptions provide alternative treatment choices for COPD, and San-Zi-Yang-Qin Decoction (SZYQD) is one of them. SZYQD is a classically effective prescription for the treatment of COPD with the effect of eliminating phlegm, relieving cough and asthma [3,4]. This prescription originally came from "*Han-Shi Yi-Tong*", written by Mao Han from Ming Dynasty. Modern pharmacological research show that Perillae Fructus can be antitussive, anti-asthmatic and anti-aging [5]; Semen Sinapis can dissolve phlegm, reduce inflammation, prevent cough and asthma [6]; Raphani Semen is able to be antioxidant, antibacterial, expectorant, also can relieve wheeze and cough [7], which are roughly consistent with their pharmacological action recorded from ancient times. Although SZYQD just consists of three herbs, there are few studies on the mechanism and pathway of SZYQD, and they cannot reflect the multi-component, multi-target and multi-pathway characteristics of traditional Chinese medicine prescription.

1.3. Characteristic of network pharmacology

Network pharmacology, based on systems biology and combined with multidirectional pharmacology, is specializing in explaining the molecular mechanism of TCM prescriptions from a more comprehensive perspective by constructing multiple network to expound relationships among drugs, molecules, targets, diseases and pathways. So it is consistent with the holistic view of treating diseases with traditional Chinese medicine [8]. Therefore, this study applies network pharmacology to preliminarily explore the molecular mechanism of SZYQD in the treatment of COPD, enlightening ideas for its in-depth research, further development and application.

1.4. The original intention of conducting this study

There are two main reasons of conducting this study with network pharmacology as follows:

Firstly, to reveal the molecular mechanism behind the curative effect. TCM prescriptions have actual therapeutic effect, but the molecular mechanism of it about how to change human pathological state has not been revealed. Through network pharmacology, SZYQD's comprehensive influence on molecules in the body can be visualized. Then according to the current research on the pathogenesis of COPD and the molecular changes seen in the course of the disease, to further investigate the specific impact of SZYQD on cells and their environment, to provide a preliminary exploration of the molecular mechanism of the prescription in the treatment of COPD.

Secondly, to provide ideas for the development of new drugs. In the past, each western medicine used to be mainly direct at single target. However, this study focused on multi-target therapy, and the possibility of developing drugs was considered at the beginning of screening active components. It can direct pharmaceutical companies in conducting in-depth research, resulting in improving the efficiency of drug research; significantly improve the success rate of drug development, reduce the cost of it; and core active components may even be extracted from SZYQD, so the utility of the prescription can be maximized.

2. Materials and methods

2.1. Materials

Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php, Version: 2. 3); UniProt (http://ctdbase.org/, updated in 2019-3-6) ; STRING Database (https://string-db.org/, Version: 11. 0) ; Cytoscape Software (version 3. 7. 0), its tool: NetworkAnalyzer, and its Apps: ClueGO,

Table 1
Potential active ingredients of SZYQD with OB and DL paraments.

No.	Potential active ingredients	OB/%	DL	Number of targets	Source
1	citrostadienol	43. 28	0. 79	1	Perillae Fructus
2	(2E, 4E, 6E)-icosa-2, 4, 6-trienoic acid	41. 64	0. 2	0	Perillae Fructus
3	(E)-(4-methylbenzylidene)-(4-phenyltriazol-1-yl)amine	57. 87	0. 19	5	Perillae Fructus
4	Stigmasterol	43. 83	0. 76	31	Perillae Fructus
5	arachidonic acid	45. 57	0. 2	38	Perillae Fructus
6	beta-carotene	37. 18	0. 58	22	Perillae Fructus
7	beta-sitosterol	36. 91	0. 75	38	Perillae Fructus
8	Spinasterol	42. 98	0. 76	3	Perillae Fructus
9	gondoic acid	30. 7	0. 2	2	Perillae Fructus
10	campest-5-en-3beta-ol	37. 58	0. 71	1	Perillae Fructus
11	2, 6, 10, 14, 18-pentamethylicosa-2, 6, 10, 14, 18-pentaene	33. 4	0. 24	1	Perillae Fructus
12	Luteolin	36. 16	0. 25	57	Perillae Fructus
13	24-methylidenelophenol	44. 19	0. 75	3	Perillae Fructus
14	CLR	37. 87	0. 68	4	Perillae Fructus
15	Cycloeucalenol	39. 73	0. 79	0	Perillae Fructus
16	Obtusifoliol	42. 55	0. 76	3	Perillae Fructus
17	Phthalic acid, butyl isohexyl ester	45. 52	0. 18	8	Perillae Fructus
18	EIC	41. 9	0. 14	15	Perillae Fructus, Sinapis Semen, Raphani Semen
19	Exceparl M-OL	31. 9	0. 16	4	Perillae Fructus, Raphani Semen
20	linolenic acid	45. 01	0. 15	16	Perillae Fructus, Sinapis Semen, Raphani Semen
21	oleic acid	33. 13	0. 14	48	Perillae Fructus, Sinapis Semen
22	Uniflex BYO	30. 13	0. 25	0	Sinapis Semen
23	2-(2-phenylethyl)-6-[[(5S, 6R, 7R, 8S)-5, 6, 7-trihydroxy-4-keto-2-(2-phenylethyl)-5, 6, 7, 8-tetrahydrochromen-8-yl]oxy]chromone	31. 31	0. 61	0	Sinapis Semen
24	Sinoacutine	63. 39	0. 53	18	Sinapis Semen
25	METHYL LINOLEATE	41. 93	0. 17	4	Raphani Semen
26	Methyllinolenate	46. 15	0. 17	6	Raphani Semen
27	icosa-8, 11, 14-trienoic acid methyl ester	44. 81	0. 23	1	Raphani Semen
28	Sitosterol	36. 91	0. 75	3	Raphani Semen
29	icosa-11, 14, 17-trienoic acid methyl ester	44. 81	0. 23	0	Raphani Semen

CluePedia; Bioconductor (https://bioconductor.org/bioLite.R) and its packages: org. Hs. eg.db, clusterProfiler (version : 3. 8. 1); The R Programming Language (RGUI) ; KEGG PATHWAY Database (https://www.kegg.jp/kegg/pathway.html, updated in 2018-10-24)

2.2. Methods

2.2.1. Collection of potential active ingredients and prediction of their targets

Oral bioavailability (OB) and Drug-likeness (DL) are two important parameters of pharmaceutically active ingredients in research and development of drug. They respectively reflect the absorption and distribution of drugs in the human body, the similarity between these ingredients and existing drugs, as well as the possibility of developing drugs. Therefore, screening the effective active ingredients and their corresponding targets of SZYQD from TCMSP under the conditions of OB > 30% and DL > 0.18 [9] (The targets data in the TCMSP database is collected from the DrugBank database). If the number of targets is insufficient, the down-regulated parameter DL is preferred in this paper. Then inputting the obtained targets into the UniProt Database, and converting "Protein names" to "Gene names" with UniProt Knowledgebase (UniProtKB).

2.2.2. Screening out action targets

In CTD, search engine "Keyword Search" is used. Choosing "Disease" and inputting "COPD", then clicking on the result, "Pulmonary diseases, Chronic Obstructive", and looking over "Genes" associated with this Disease. Which are included in them, those targets of the above are regarded as action targets of SZYQD in the treatment of COPD. Afterward, inputting "Gene names" of action targets into the STRING Database, using its tool "Multiple proteins search", the obtained protein-protein interaction (PPI) relationship is saved as a file in TSV format.

2.2.3. Construction of the "drugs-components-action targets" network and PPI network

Then the action targets and their corresponding active ingredients with their correlative herbs are input into Cytoscape software to construct the "Drugs-components-targets" network. In addition, loading information of column "node1","node2" and "combined score" in the file in TSV format above into Cytoscape software to get an initial PPI network. Analyzing it with the tool Network Analyzer and mainly paying attention to one of its statistic results "Degree", according to which, editing the final network and exporting it to the image.

2.2.4. Enrichment of gene ontology (GO) function and KEGG pathway

RGUI and org. Hs. eg.db are applied to obtain entrezIDs of action targets. Based on which, RGUI and clusterProfiler are used to enrich GO function, including MolecularFunction (MF) 、BiologicalProcess (BP) and CellularComponent (CC) ; Moreover, typing action targets into ClueGO to enrich GO function (BP only) and KEGG pathway; editing the networks with CluePedia so that they are capable of showing the connection between action targets and pathways; and meanwhile, manually screening out nodes as needed. Finally, entrezIDs are entered into KEGG PATHWAY Database to highlight action targets on the pictures of correlative pathways.

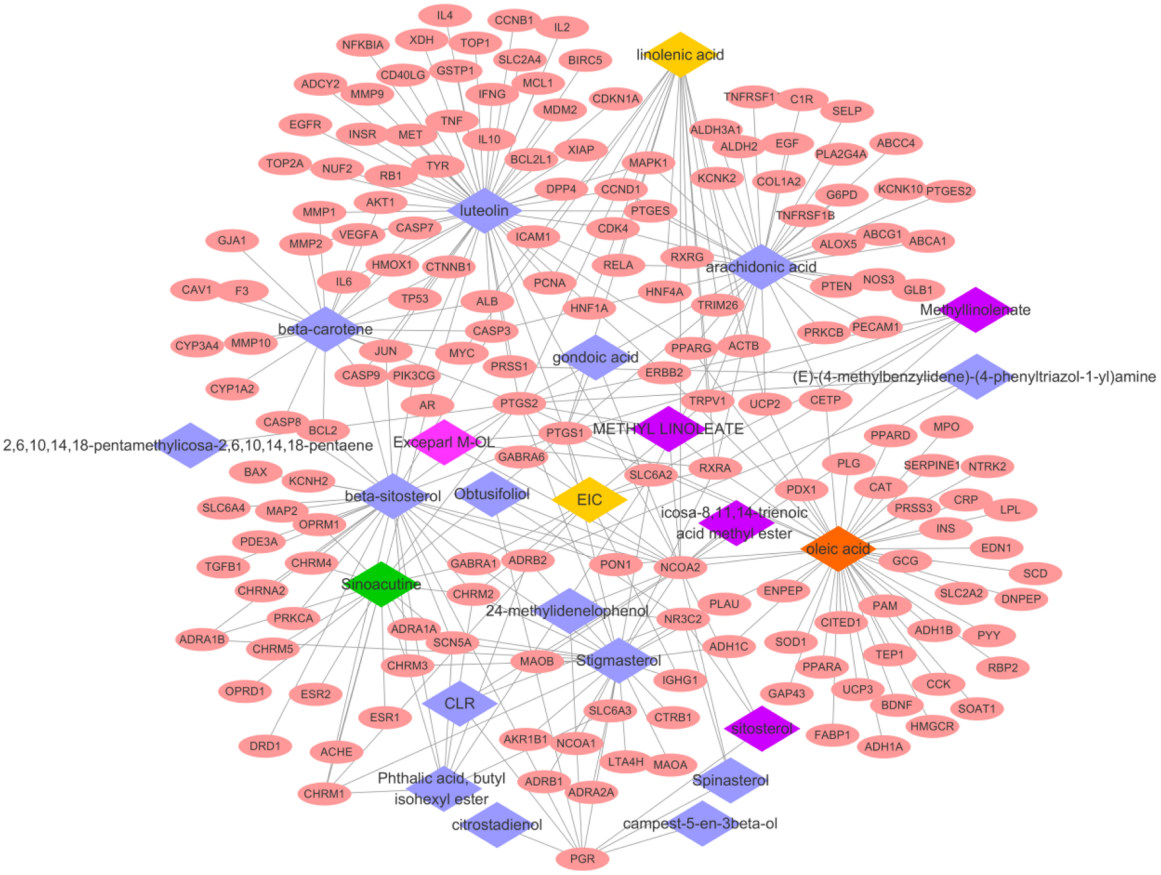

Fig. 1. "Drugs-Components-Action targets" network of SZYQD.

Table 2
Information of action targets from potential active ingredients of SZYQD (Degreed ≥ 27).

No.	Uniprot ID	Gene name	Protein name	Degree
1	P01308	INS	Insulin	115
2	P31749	AKT1	RAC-alpha serine/threonine-protein kinase	107
3	P02768	ALB	Serum albumin	106
4	P05231	IL6	Interleukin-6	93
5	P15692	VEGFA	Vascular endothelial growth factor A	86
6	P04637	TP53	Cellular tumor antigen p53	84
7	P01375	TNF	Tumor necrosis factor	84
8	P42574	CASP3	Caspase-3	82
9	P28482	MAPK1	Mitogen-activated protein kinase 1	80
10	P05412	JUN	Transcription factor AP-1	79
11	P01133	EGF	Pro-epidermal growth factor	78
12	P00533	EGFR	Epidermal growth factor receptor	76
13	P35354	PTGS2	Prostaglandin G/H synthase 2	75
14	P14780	MMP9	Matrix metalloproteinase-9	72
15	P01106	MYC	Myc proto-oncogene protein	71
16	P04040	CAT	Catalase	69
17	P29474	NOS3	Nitric oxide synthase, endothelial	65
18	P37231	PPARG	Peroxisome proliferator-activated receptor gamma	65
19	P23560	BDNF	Brain-derived neurotrophic factor	65
20	P03372	ESR1	Estrogen receptor	63
21	P24385	CCND1	G1/S-specific cyclin-D1	61
22	P60484	PTEN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	61
23	P08253	MMP2	72 kDa type IV collagenase	61
24	P04626	ERBB2	Receptor tyrosine-protein kinase erbB-2	59
25	P60709	ACTB	Actin, cytoplasmic 1	59
26	P05305	EDN1	Endothelin-1	58
27	P22301	IL10	Interleukin-10	56
28	P35222	CTNNB1	Catenin beta-1	55
29	P05362	ICAM1	Intercellular adhesion molecule 1	54
30	Q04206	RELA	Transcription factor p65	53
31	Q07817	BCL2L1	Bcl-2-like protein 1	52
32	Q14790	CASP8	Caspase-8	51
33	P05121	SERPINE1	Plasminogen activator inhibitor 1	50
34	P05112	IL4	Interleukin-4	50
35	P02741	CRP	C-reactive protein	50
36	P10275	AR	Androgen receptor	48
37	Q03135	CAV1	Caveolin-1	48
38	P01137	TGFB1	Transforming growth factor beta-1	47
39	P09601	HMOX1	Heme oxygenase 1	47
40	P55211	CASP9	Caspase-9	46
41	P60568	IL2	Interleukin-2	46
42	P05164	MPO	Myeloperoxidase	45
43	P38936	CDKN1A	Cyclin-dependent kinase inhibitor 1	44
44	P16284	PECAM1	Platelet endothelial cell adhesion molecule	44
45	Q07820	MCL1	Induced myeloid leukemia cell differentiation protein Mcl-1	43
46	P01579	IFNG	Interferon gamma	43
47	Q00987	MDM2	E3 ubiquitin-protein ligase Mdm2	42
48	P11802	CDK4	Cell division protein kinase 4	40
49	P01275	GCG	Glucagon	40
50	P00747	PLG	Plasminogen	39
51	P06401	PGR	Progesterone receptor	39
52	P03956	MMP1	Interstitial collagenase	39
53	P19438	TNFRSF1A	Tumor necrosis factor receptor superfamily member 1A	38
54	P14635	CCNB1	G2/mitotic-specific cyclin-B1	38
55	P98170	XIAP	Baculoviral IAP repeat-containing protein 4	35
56	P00441	SOD1	Superoxide dismutase [Cu-Zn]	35
57	Q07869	PPARA	Peroxisome proliferator-activated receptor alpha	35
58	P25963	NFKBIA	NF-kappa-B inhibitor alpha	34
59	P29965	CD40LG	CD40 ligand	34
60	P06858	LPL	Lipoprotein lipase	33
61	P14672	SLC2A4	Solute carrier family 2, facilitated glucose transporter member 4	33
62	P06400	RB1	Retinoblastoma-associated protein	32
63	P08581	MET	Hepatocyte growth factor receptor	32
64	P41235	HNF4A	Hepatocyte nuclear factor 4-alpha	32
65	P17252	PRKCA	Protein kinase C alpha type	32
66	P00749	PLAU	Urokinase-type plasminogen activator	31
67	P07550	ADRB2	Beta-2 adrenergic receptor	31
68	P22303	ACHE	Acetylcholinesterase	31
69	Q8NER1	TRPV1(VR1)	Transient receptor potential cation channel subfamily V member 1	31
70	P17302	GJA1	Gap junction alpha-1 protein	30
71	P08684	CYP3A4	Cytochrome P450 3A4	29
72	P16109	SELP	P-selectin	29
73	P55210	CASP7	Caspase-7	28
74	P27487	DPP4	Dipeptidyl peptidase IV	28
75	P06307	CCK	Cholecystokinin	27
76	O95477	ABCA1	ATP-binding cassette sub-family A member 1	27
77	P13726	F3	Tissue factor	27
78	P09211	GSTP1	Glutathione S-transferase P	27

3. Results

3.1. The information of potential active ingredients and their targets in SZYQD

A total of 231 active ingredients and 1451 targets were shown when input typing Perillae Fructus, Sinapis Semen, and Raphani Semen into TCMSP. Due to insufficient targets under DL > 0.18, the new condition, OB > 30% and DL > 0.14, was set after literature consulting on ingredients under OB > 30%, Further screening showed that the number of potential active ingredients of Perillae Fructus, Sinapis Semen and Raphani Semen were 21, 6 and 8, respectively (Table 1), which were mainly phytosterols and fatty acids. After removing duplicate targets, there were 186 targets in total.

In UniProt Database, search engine “UniProtKB” was used and then downloading the data filtered by “Reviewed (Swiss-Prot)” and “Human” in Excel format. Adding a filter for the column "Protein names" in the Excel file, and selecting "Contains" in the "Text Filters", into which entering the 186 targets mentioned above and further checking them one by one. Eventually, only 170 names of targets were successfully converted, which were all related to COPD by looking through “Genes” associated with this Disease in CTD and they were named as action targets.

3.2. Construction of networks

Entering 170 action targets into STRING Database and limiting the organism to “Homo sapiens”. Clicking “SEARCH” and the selected proteins were checked manually in the following web page. On account of the unrecognized target “IGHG1”, an initial PPI network was obtained containing only 169 targets.

Then 170 action targets and their corresponding active ingredients with their correlative herbs were imported into Cytoscape software to construct the “Drugs-components-targets” network (Fig. 1). In addition, the initial PPI network and its combined scores were imported into Cytoscape software. According to the Degree of targets (Table 2), the final PPI network was constructed (Fig. 2).

3.3. Analysis of networks

“Drugs-Components-Action targets” network of SZYQD (Fig. 1) contains 194 nodes and 302 edges in total. Diamond-shaped nodes represent active ingredients (among them, a total of 24 diamond-shaped nodes only show the ones which have targets. Blue, purple and green diamond-shaped nodes represent the ones of Perillae Fructus, Raphani

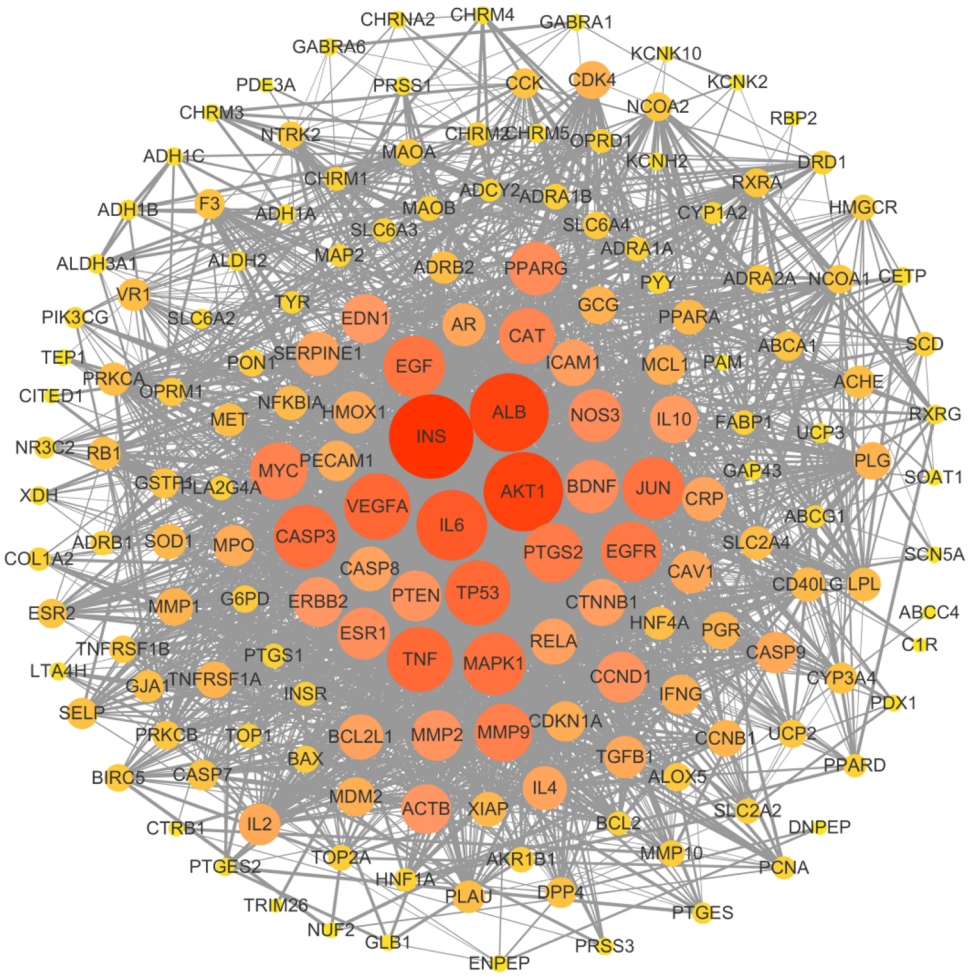

Fig. 2. PPI network of action targets in SZYQD.

Semen and Sinapis Semen respectively; yellow diamond-shaped nodes represent the same ones of the three herbs; burgundy diamond-shaped nodes represent the same ones of Perillae Fructus and Raphani Semen, red diamond-shaped nodes represent the same ones of Perillae Fructus and Sinapis Semen). The pink elliptic nodes represent 170 action targets and the edges represent the relationship between active ingredients and action targets.

PPI network of action targets in SZYQD (Fig. 2) contains 169 nodes and 2620 edges. Nodes represent proteins, edges represent interaction of proteins. The size and color of the nodes based on Degree (Degree ranges from 1 to 115, among which, the target VR1 ranks 70, Degree = 30). When the node is bigger and the color of it is redder, the Degree of the protein is larger. The thickness of the edge based on Combined score, and the thicker the edge is, the greater the Combined score is.

3.4. Analysis of the enrichment of GO function and KEGG pathway

3.4.1. Analysis of the enrichment of GO function

Applying RGUI and clusterProfiler [10] to enrich the GO function of 170 action targets ($pV < 0.05$, $qV < 0.05$. EntrezIDs of action targets are shown in Table 4. The abscissa represents the number of targets involved in each term; and the color of terms is from red to blue, indicating that the p.adjust becomes larger, which means that the term is more significant if it is redder).The results were GO-MF, GO-BP and GO-CC respectively as follow:

GO-MF enrichment contains 177 terms and Fig. 3 depicts the most significant 30 ones, including steroid hormone receptor activity, nuclear receptor activity, transcription factor activity, protein heterodimerization activity, G-protein coupled amine receptor activity, steroid binding, phosphatase binding, protein phosphatase binding, acetylcholine receptor activity, protease binding, neurotransmitter receptor activity, fatty acid binding, cofactor binding, adrenergic receptor activity, serine hydrolase activity, catecholamine binding, antioxidant activity, death domain binding, heme binding, activating transcription factor binding, nitric-oxide synthase binding, etc, which are involved in regulating the cell proliferation, growth and apoptosis; gene transcription and expression; protein synthesis, activation and decomposition; utilization of energy; regulation of sympathetic and parasympathetic nerve transmission; providing anti-oxidation protection, regulating immune system, dilating blood vessels and so on.

GO-BP enrichment contains 2072 terms and Fig. 4 shows the most prominent 30 ones, including response to extracellular stimulus, response to steroid hormone, response to lipopolysaccharide, vascular process in circulatory system, regulation of blood vessel diameter, response to molecule of bacterial origin, muscle cell proliferation, smooth

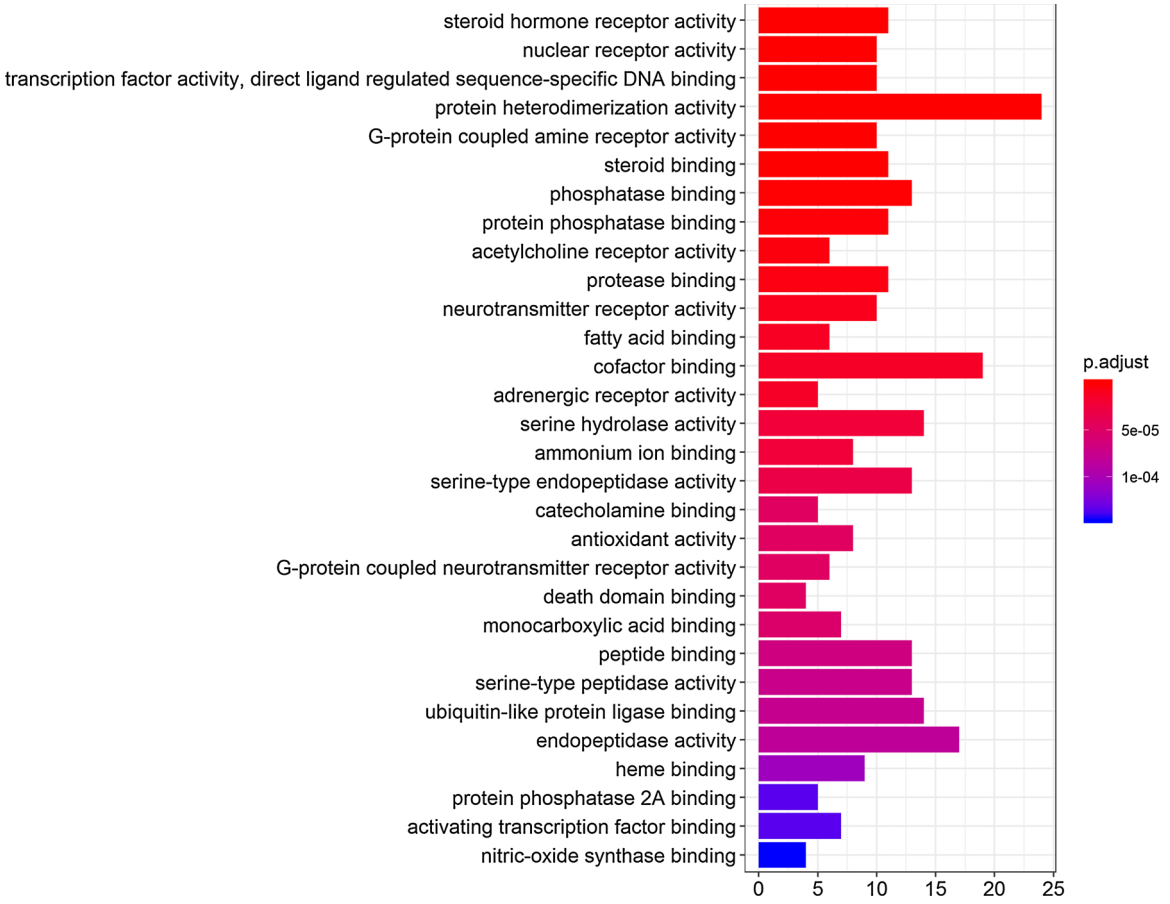

Fig. 3. Molecular function results of GO enrichment analysis (GO-MF, from cluster profiler).

muscle cell proliferation, response to hypoxia, response to reactive oxygen species, response to oxidative stress, extrinsic apoptotic signaling pathway, etc.

GO-CC enrichment contains 71 terms and Fig. 5 shows the most significant 30 ones, including membrane raft, membrane microdomain, membrane region, caveola, plasma membrane raft, vesicle lumen, secretory granule lumen, cytoplasmic vesicle lumen, dendrite membrane, platelet alpha granule, receptor complex, external side of plasma membrane, postsynaptic membrane, neuron projection membrane, cyclin-dependent protein kinase holoenzyme complex, synaptic membrane, mitochondrial outer membrane, blood microparticle, neuronal cell body, organelle outer membrane, RNA polymerase II transcription factor complex, protein kinase complex, nuclear transcription factor complex, etc.

Moreover, typing 170 action targets into ClueGO to enrich GO function (BP only, Use GO Term Fusion, pV ≤ 0. 05) and editing the networks with CluePedia. The enrichment contains 695 terms. In order to further understand the antioxidant effect of SZYQD and the targets involved, screening out terms which contains “oxygen” and “oxi-” manually (Fig. 6, the size and color of the nodes are ignored here; Table 3, the associated targets are action targets associated with corresponding GO terms), and there are a total of 36 terms, including response to reactive oxygen species, response to oxygen levels, response to oxygen-containing compound, regulation of reactive oxygen species biosynthetic process, regulation of reactive oxygen species metabolic process, superoxide metabolic process, response to toxic substance, removal of superoxide radicals, hydrogen peroxide metabolic process, regulation of oxidoreductase activity and so on.

3.4.2. Analysis of the enrichment of KEGG pathway

Typing 170 action targets into ClueGO to enrich KEGG pathway (pV < 0. 05) and editing the networks with CluePedia. The enrichment contains 111 terms and only 43 ones were retained by removing ones belonging to other diseases manually (Fig. 7, the size and color of the nodes are ignored here), including the Cholinergic synapse, Th17 cell differentiation, Fluid shear stress and atherosclerosis, Apoptosis, PI3K-Akt signaling pathway, NF-κB signaling pathway, TNF signaling pathway, P53, C-type lectin receptor signaling pathway and so on.

To further explore the 170 action targets of SZYQD in related pathways and their interrelationships, entering their entrezIDs into KEGG PATHWAY Database to highlight action targets on the pictures of correlative pathways with the tool “Search & Color Pathway” (15 targets are unrecognized, which was recorded in Table 4). After repeated comparison and screening, the results were included, as shown in Figs. 8–9, and the action targets were orange ones.

4. Discussion

SZYQD originally came from “*Han-Shi Yi-Tong*”, which is a classic and basic prescription in the treatment of COPD [11]. Based on the method of network pharmacology, its molecular mechanism in the treatment of COPD is explored here.

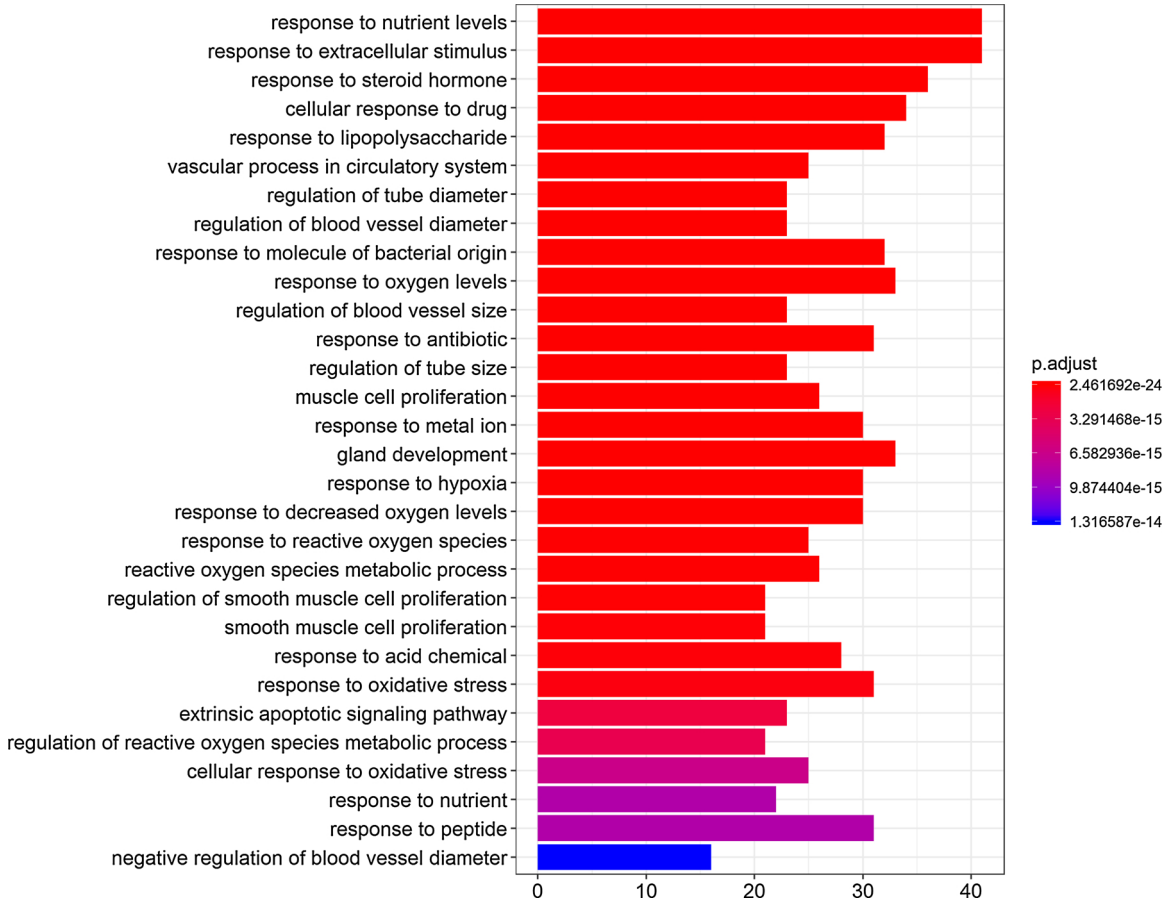

Fig. 4. Biological process results of GO enrichment analysis (GO-BP, from cluster profiler).

4.1. Modern pharmacological research on relevant potential active ingredients

Alpha-linolenic acid, the same ingredient of Perillae Fructus, Sinapis Semen and Raphani Semen, can reduce inflammation, scavenge oxygen radical and other toxin, regulate imbalance of cell proliferation-apoptosis, which makes it effectively protect mice from LPS-induced acute lung injury (ALI), by reducing the number of inflammatory cells, the expression of TNF-α and IL-6 in the bronchoalveolar lavage fluid; down-regulating the expression MPO, MDA, NF-κB and IκB in lung tissue; increasing the expression of caspase-3, SOD and GSH in lung tissue, etc [12]. Oleic acid, the same ingredient of Perillae Fructus and Sinapis Semen, is good at inhibiting inflammation, modulating leukocytes activity, enhancing bactericidal and fungicidal, etc [13]. Beta-sitosterol and Stigmasterol of Perillae Fructushave have anti-inflammatory effect [14]; the luteolin is able to inhibit bacteria and virus, attenuate smooth muscle cell proliferation and response to inflammation, by inhibiting the phosphorylation of AKT, down-regulating the expressions of TNF-α, COX2, NF-κ B, etc [15]; the beta-carotene part can activate caspase-8 and other factors to induce apoptosis, decrease the expression of COX2 and Bcl-2 to prevent abnormal cell proliferation, regulate NF-κB pathway, inhibit AP-1, etc [16]. METHYL LINOLEATE of Raphani Semen can relieve the ear swelling of mice with croton-oil-induced acute inflammation and the inflammatory response of rats with Complete-Freund's-Adjuvant-induced chronic arthritis [17].

4.2. The connotation of methods

"Drugs-Components-Action targets" network of SZYQD embodies multi-component (potential active ingredients) and multi-target in the treatment of COPD, which are the characteristics of TCM (Fig. 1). PPI network of action targets in SZYQD shows how these targets are connected to each other, including their strength and number of the connections (Fig. 2). The enrichment of GO function reflects the significant function of action targets themselves and the connections of them in multiple parts of human body, including cells and their living environment (Fig. 3–6). The enrichment of KEGG pathway shows the location of action targets in each pathway, and the potential active ingredients affect the overall function of these pathways by affecting the expression or function of these targets(Figs. 7–9).

4.3. Analyzing molecular mechanism of SZYQD based on pathogenesis of COPD

Oxidative stress plays an important role in the pathogenesis and exacerbation of COPD [18]. SOD1, CAT, HMOX1, GSTP1 and so on, have the effect of anti-oxidation and elimination of harmful substances in the human body. This study predicts that SZYQD can remove the toxic substances in the body and regulate the imbalance of oxidation-antioxidation in COPD patients, by enhancing the expression or activation of these antioxidants.

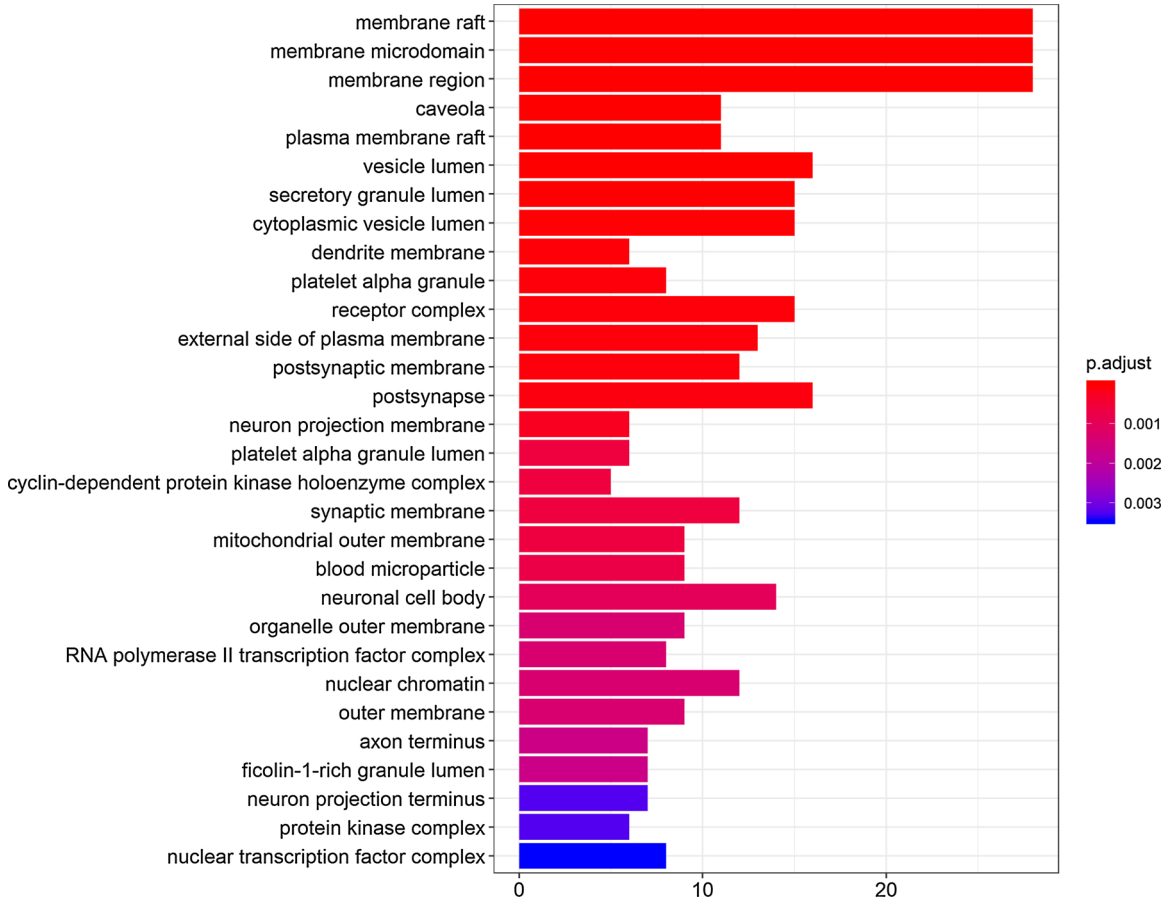

Fig. 5. Cellular component results of GO enrichment analysis (GO-CC, from cluster profiler).

PI3K-AKT signaling pathway (Fig. 8) can be activated by oxidative stress, which is an important factor in the pathogenesis of COPD [19]. Research presented that the expressions of PI3K mRNA and AKT1 mRNA in the lung tissue of COPD rat models were markedly increased [20]; PI3K inhibitors could protect them from chronic lung injury induced by Trypsin, inhibit inflammatory reaction and edema, and relieve pulmonary emphysema and airway remodeling [21]; Cyclin D1 (CCND1) significantly increased in the lung tissue of smoke-induced COPD rats, compared with that of the normal group [22]; in COPD rat models which were stimulated by LPS and smoke, the expression of Bcl-2 in the lung tissue was significantly increased [23], the expression of C-myc significantly increased in trachea and lung tissue, especially in fibroblasts and smooth muscle cells [24]; the levels of Bcl-xl mRNA and Mcl-1 mRNA in neutrophilic granulocytes in peripheral blood of patients with mild or moderate COPD were markedly up-regulated compared with those of healthy people [25]; the important role of ERK in the pathogenesis of COPD have been demonstrated by multiple studies, inhibition of ERK phosphorylation can help attenuate the inflammatory response, regulate the imbalance between oxidative and antioxidant stress, as well as apoptosis of smooth muscle cells, and increase the apoptosis of neutrophilic granulocytes, etc [26]; in the smoking group and COPD group, compared with the normal group, the pulmonary arterial wall was thicker and the lumen was narrower, and the positive reaction of PKC-α and Cyclin D1 in the cytoplasm of smooth muscle cells were stronger, suggesting the two proteins may be involved in the proliferation of smooth muscle cells in the pulmonary arteriole caused by smoking [27]. This study predicts that SZYQD may inhibit the abnormal proliferation and postponent survival of cells by inhibiting the stimulation of cytokines upstream of this pathway, the activity of kinases such as PI3K and AKT1, and the expression of Bcl-2 and C-myc.

As nuclear transcription factor, NF-κB plays an important role in the pathogenesis of COPD [28]. Research has reported that compared with a healthy population, cytoplasm protein IκB in lung tissue significantly decreased, and the expression of p-IκB and karyon protein NF-κB p65 were up-regulated in the LPS-smoke-induced COPD rat models [29]. Activation of NF-κB can induce differentiation of Th17 cells and up-regulation of cytokines, adhesion molecules, chemokines, vasoactive regulators and proteases downstream in C-type lectin receptor, IL-17, NF-κB and TNF signaling pathway (Fig. 9),etc, further stimulating the proliferation and differentiation of inflammatory cells and thereby expanding the inflammatory response. Previous research found that smoke or LPS-smoke induced COPD rat models compared with the placebo-treated group, in the lung tissue, the expression of IL-4 and IFN-γ prominently decreased [30], MMP1 [31]and TGF-β1 [32] significantly increased; ICAM-1 in the lung tissue of bronchus, IL-2 in bronchoalveolar lavage fluid [33] and MMP9 in serum [34] increased markedly, IL-4 and IL-10 decreased significantly [30].Scholars found that, compared with the healthy control group, the expression of pulmonary vascular eNOS in the COPD rat models induced by smoke and PPE prominently decreased [35]; In addition, compared with the

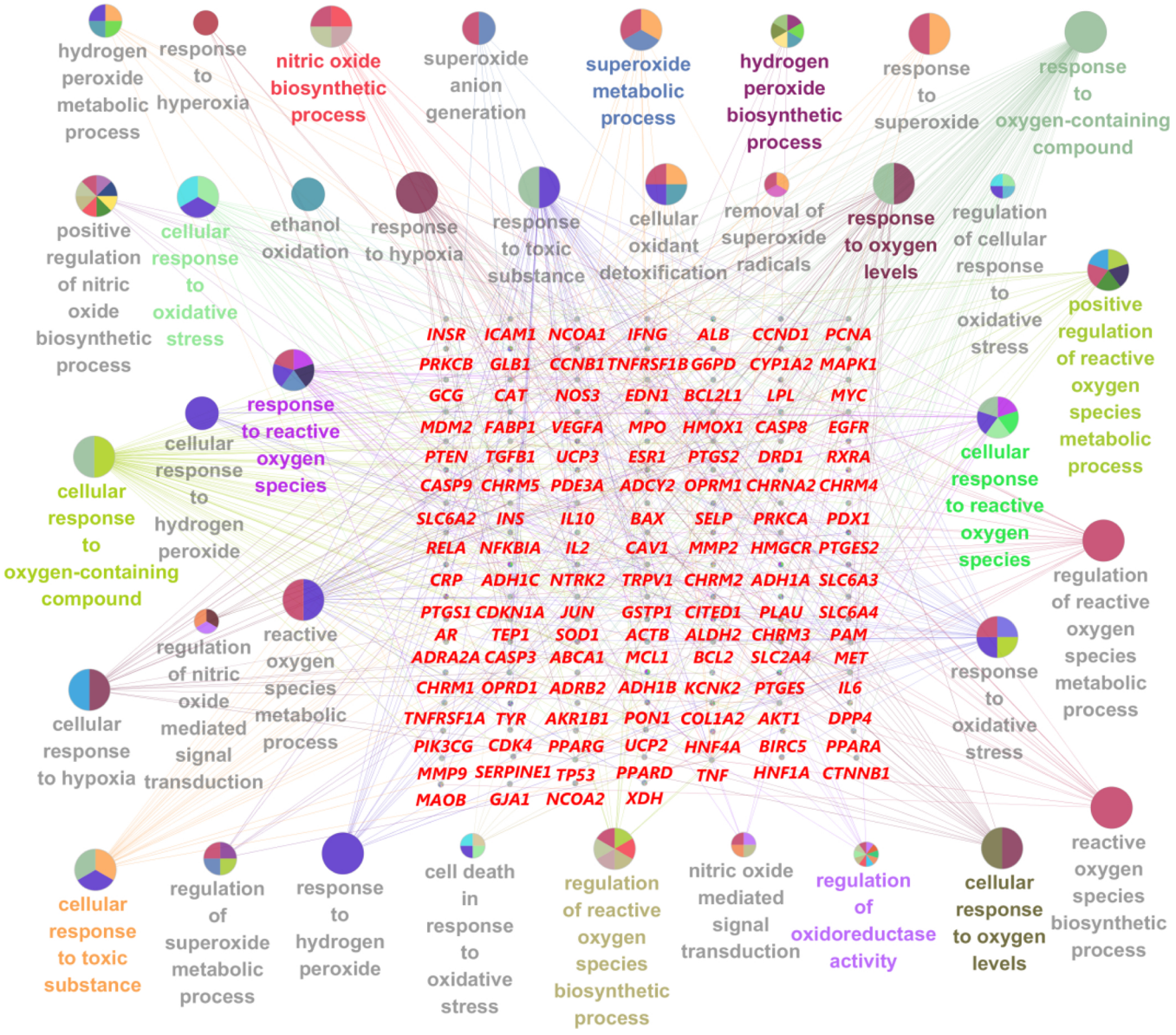

Fig. 6. Selected biological process results of GO enrichment analysis (from Clue GO).

normal control group, the expressions of MMP2 and COX2 in the induced sputum of COPD patients at all levels were significantly up-regulated [36], and the concentration of IL-6 in the serum was markedly up-regulated [37]. This study conjectures that through comprehensive regulation of the above molecules and etc, SZYQD may make contribution to prevent excessive production of cytokines; decomposition and destruction of tissues by protease; the proliferation, chemotaxis and adhesion of inflammatory cells; angiectasis, atherosclerosis and so on.

Apoptosis may be one of the pathogenesis of COPD [18]. Research have indicated that in the lung tissue [38] and serum [39] of patients with COPD, the expression of p53 increased obviously; and experimental results presented that in LPS-smoke induced COPD rat models compared with the normal group, the number of apoptotic alveolar epithelial cells and apoptotic endothelial cells increased, p53 in the lung tissue increased, and both were positively correlated, p53 inhibitors can reduce the level of them [40]. Research has documented that the number of apoptotic cells and the expression of caspase-3 in bronchoalveolar lavage fluid of COPD patients significantly increased compared with the healthy people [41]. Intervening human pulmonary artery endothelial cells cultured in vitro with 10% of cigarette smoke extract (CSE), compared with normal control group, cell survival rate decreased evidently after 12 h, the activation of caspase-3, caspase-8 and caspase-9 rose significantly after 2 h; but if before the intervention, with 20 μg/ml TNF alpha inhibitors, the pretreatment of cells could effectively resist the effect of the former [42]. Research showed that the positive rate of Bax, Bcl-2 in alveolar wall cells and Bax / Bcl-2 in alveolar wall cells were observably rised in smokers with COPD or not, in

Table 3
Associated targets of selected biological process results of GO enrichment analysis(from ClueGO).

No.	GO Term	Associated Targets
1	response to reactive oxygen species	[AKT1, BCL2, CASP3, CAT, CDKN1A, EDN1, EGFR, FABP1, GSTP1, HMOX1, IL10, IL6, JUN, MAPK1, MDM2, MET, MMP9, MPO, NCOA1, NOS3, PCNA, RELA, SLC6A2, SOD1, TNF, UCP2, UCP3]
2	cellular response to reactive oxygen species	[AKT1, CDKN1A, EGFR, FABP1, IL10, IL6, JUN, MAPK1, MDM2, MET, MMP9, MPO, NCOA1, NOS3, PCNA, RELA, SLC6A2, SOD1, TNF]
3	response to oxygen levels	[AKT1, BCL2, CASP3, CAT, CAV1, CCNB1, CDK4, CDKN1A, DPP4, EDN1, FABP1, HMOX1, ICAM1, KCNK2, MDM2, MMP2, MYC, NCOA1, OPRD1, PAM, PLAU, PPARA, PPARD, PPARG, PTEN, PTGS2, SLC2A4, SLC6A4, TEP1, TGFB1, TP53, UCP2, UCP3, VEGFA]
4	cellular response to oxygen levels	[AKT1, BCL2, CAV1, CCNB1, EDN1, FABP1, HMOX1, ICAM1, KCNK2, MDM2, MYC, NCOA1, OPRD1, PPARD, PPARG, PTEN, PTGS2, SLC2A4, TEP1, TP53, VEGFA]
5	reactive oxygen species metabolic process	[AKT1, BCL2, CAT, CAV1, CDKN1A, CRP, CYP1A2, EDN1, EGFR, G6PD, GSTP1, ICAM1, IFNG, IL10, INS, INSR, MAOB, MPO, NOS3, PTGES2, PTGS2, SOD1, TGFB1, TNF, TP53, TRPV1, XDH]
6	response to oxygen-containing compound	[ABCA1, ACTB, ADCY2, ADRA2A, ADRB2, AKR1B1, AKT1, AR, BCL2, BCL2L1, BIRC5, CASP3, CASP8, CASP9, CAT, CAV1, CCNB1, CCND1, CDK4, CDKN1A, CHRM1, CHRM2, CHRM3, CHRM4, CHRM5, CITED1, COL1A2, CTNNB1, CYP1A2, DRD1, EDN1, EGFR, ESR1, FABP1, G6PD, GCG, GJA1, GLB1, GSTP1, HMGCR, HMOX1, HNF1A, HNF4A, ICAM1, IL10, IL2, IL6, INS, INSR, JUN, LPL, MAOB, MAPK1, MDM2, MET, MMP2, MMP9, MPO, NCOA1, NCOA2, NFKBIA, NOS3, NTRK2, OPRM1, PAM, PCNA, PDE3A, PDX1, PIK3CG, PON1, PPARA, PPARD, PPARG, PRKCA, PRKCB, PTEN, PTGES, PTGS2, RELA, RXRA, SELP, SERPINE1, SLC2A4, SLC6A2, SLC6A3, SLC6A4, SOD1, TEP1, TGFB1, TNF, TNFRSF1A, TNFRSF1B, TP53, TRPV1, TYR, UCP2, UCP3]
7	cellular response to oxygen-containing compound	[ABCA1, ACTB, ADCY2, ADRA2A, ADRB2, AKR1B1, AKT1, AR, BCL2L1, BIRC5, CASP9, CAV1, CCNB1, CDK4, CDKN1A, CHRM1, CHRM2, CHRM3, CHRM4, CHRM5, COL1A2, CTNNB1, DRD1, EDN1, EGFR, ESR1, FABP1, GCG, GSTP1, HMGCR, ICAM1, IL10, IL6, INS, INSR, JUN, MAPK1, MDM2, MET, MMP2, MMP9, MPO, NCOA1, NFKBIA, NOS3, NTRK2, PCNA, PDE3A, PDX1, PIK3CG, PPARD, PPARG, PRKCA, PRKCB, PTEN, PTGS2, RELA, SERPINE1, SLC2A4, SLC6A2, SLC6A4, SOD1, TEP1, TGFB1, TNF, TNFRSF1B, TP53, TRPV1, UCP2]
8	reactive oxygen species biosynthetic process	[AKT1, CAV1, CYP1A2, EDN1, ICAM1, IFNG, IL10, INS, INSR, MAOB, MPO, NOS3, PTGES2, PTGS2, SOD1, TNF, TRPV1]
9	regulation of reactive oxygen species biosynthetic process	[AKT1, CAV1, EDN1, ICAM1, IFNG, IL10, INS, INSR, PTGS2, TNF, TRPV1]
10	regulation of reactive oxygen species metabolic process	[AKT1, BCL2, CAV1, CDKN1A, CRP, EDN1, EGFR, G6PD, GSTP1, ICAM1, IFNG, IL10, INS, INSR, PTGS2, SOD1, TGFB1, TNF, TP53, TRPV1, XDH]
11	positive regulation of reactive oxygen species metabolic process	[AKT1, CDKN1A, CRP, EDN1, EGFR, GSTP1, ICAM1, IFNG, INSR, PTGS2, SOD1, TGFB1, TNF, TP53, TRPV1, XDH]
12	response to superoxide	[MPO, NOS3, SOD1, TNF, UCP2, UCP3]
13	response to hypoxia	[AKT1, BCL2, CASP3, CAT, CAV1, CCNB1, DPP4, EDN1, FABP1, HMOX1, ICAM1, KCNK2, MDM2, MMP2, MYC, NCOA1, OPRD1, PAM, PLAU, PPARA, PPARD, PTEN, PTGS2, SLC2A4, SLC6A4, TEP1, TGFB1, TP53, UCP2, UCP3, VEGFA]
14	ethanol oxidation	[ADH1A, ADH1B, ADH1C, ALDH2]
15	superoxide metabolic process	[CRP, EDN1, EGFR, GSTP1, MPO, NOS3, SOD1, TGFB1, TNF]
16	nitric oxide biosynthetic process	[AKT1, CAV1, EDN1, ICAM1, IFNG, IL10, INSR, NOS3, PTGS2, TNF, TRPV1]
17	response to oxidative stress	[AKT1, BCL2, CASP3, CAT, CDKN1A, EDN1, EGFR, FABP1, G6PD, GSTP1, HMOX1, HNF1A, IL10, IL6, INS, JUN, MAPK1, MCL1, MDM2, MET, MMP9, MPO, NCOA1, NOS3, PCNA, PTGS1, PTGS2, RELA, SELP, SLC6A2, SOD1, TNF, TP53, UCP2, UCP3]
18	nitric oxide mediated signal transduction	[EGFR, INS, NOS3, PDX1, VEGFA]
19	response to toxic substance	[ACTB, ALB, BAX, BCL2, BCL2L1, CASP3, CASP8, CAT, CCNB1, CCND1, CDK4, CDKN1A, CHRNA2, DRD1, EDN1, FABP1, G6PD, GSTP1, HMGCR, HMOX1, ICAM1, IL10, IL2, IL6, JUN, MAOB, MAPK1, MDM2, MET, MPO, NCOA1, NOS3, OPRD1, OPRM1, PCNA, PON1, PPARA, PTEN, PTGS1, PTGS2, RELA, SLC2A4, SLC6A2, SLC6A3, SLC6A4, SOD1, TEP1, TNF]
20	regulation of nitric oxide mediated signal transduction	[EGFR, INS, VEGFA]
21	removal of superoxide radicals	[MPO, NOS3, SOD1, TNF]
22	cellular response to oxidative stress	[AKT1, BCL2, CAT, CDKN1A, EGFR, FABP1, G6PD, HMOX1, IL10, IL6, INS, JUN, MAPK1, MCL1, MDM2, MET, MMP9, MPO, NCOA1, NOS3, PCNA, RELA, SLC6A2, SOD1, TNF, TP53]
23	cell death in response to oxidative stress	[AKT1, BCL2, IL10, IL6, INS, MCL1, MET, SOD1]
24	response to hydrogen peroxide	[BCL2, CASP3, CAT, CDKN1A, FABP1, HMOX1, IL10, IL6, JUN, MDM2, MET, NCOA1, PCNA, RELA, SLC6A2, SOD1]
25	superoxide anion generation	[CRP, EDN1, EGFR, GSTP1, SOD1, TGFB1]
26	hydrogen peroxide metabolic process	[CAT, CYP1A2, EGFR, MAOB, MPO, PTGS2, SOD1]
27	positive regulation of nitric oxide biosynthetic process	[AKT1, EDN1, ICAM1, IFNG, INSR, PTGS2, TNF, TRPV1]
28	hydrogen peroxide biosynthetic process	[CYP1A2, MAOB, PTGS2, SOD1]
29	regulation of oxidoreductase activity	[AKT1, CAV1, EDN1, EGFR, IFNG, INS, NOS3, TNF]
30	response to hyperoxia	[CAT, CAV1, CDK4, CDKN1A, PPARG]
31	cellular response to hydrogen peroxide	[CDKN1A, FABP1, IL10, IL6, MDM2, MET, PCNA, RELA, SLC6A2]
32	cellular response to hypoxia	[AKT1, BCL2, CCNB1, EDN1, FABP1, HMOX1, ICAM1, KCNK2, MDM2, MYC, NCOA1, OPRD1, PPARD, PTEN, PTGS2, SLC2A4, TEP1, TP53, VEGFA]
33	regulation of superoxide metabolic process	[CRP, EGFR, GSTP1, SOD1, TGFB1, TNF]
34	cellular response to toxic substance	[ACTB, ALB, CAT, CDKN1A, FABP1, GSTP1, HMOX1, IL10, IL6, MDM2, MET, MPO, NOS3, OPRD1, PCNA, PTEN, PTGS1, PTGS2, RELA, SLC6A2, SOD1, TEP1, TNF]
35	cellular oxidant detoxification	[ALB, CAT, FABP1, GSTP1, MPO, NOS3, PTGS1, PTGS2, SOD1, TNF]
36	regulation of cellular response to oxidative stress	[AKT1, IL10, IL6, INS, MCL1, MET, SOD1, TNF]

Table 4
Action targets from potential active ingredients of SZYQD.

H	entrez ID	Gene name	entrez ID	Gene name	entrez ID	Gene name	entrez ID
PGR	5241	ALOX5	240	PDE3A	5139	MET	4233
PTGS2	5743	PLA2G4A	5321	CHRNA2	1135	ESR1	2099
ADRB2	154	PTEN	5728	SLC6A4	6532	CHRM5	1133
MAOB	4129	SELP	6403	OPRM1	4988	OPRD1	4985
PRSS1	5644	PTGES	9536	BAX	581	ACHE	43
NR3C2	4306	GLB1	2720	PRKCA	5578	ESR2	2100
NCOA2	10499	ALDH2	217	TGFB1	7040	GABRA6	2559
ADH1C	126	ABCA1	19	FABP1	2168	HNF4A	3172
IGHG1	3500	ALDH3A1	218	RBP2	5948	HNF1A	6927
RXRA	6256	GCG	2641	AR	367	ACTB	60
NCOA1	8648	C1R	715	DPP4	1803	PLG	5340
PTGS1	5742	CETP	1071	EGFR	1956	ADH1B	125
ADRA2A	150	ABCG1	9619	BCL2L1	598	ADH1A	124
SLC6A2	6530	ABCC4	10257	CDKN1A	1026	PRSS3	5646
SLC6A3	6531	KCNK10	54207	MMP9	4318	SOD1	6647
AKR1B1	231	TNFRSF1B	7133	IL10	3586	CAT	847
PLAU	5328	PTGES2	80142	RB1	5925	INS	3630
LTA4H	4048	KCNK2	3776	TNF	7124	EDN1	1906
MAOA	4128	COL1A2	1278	IL6	3569	LPL	4023
CTRB1	1504	AKT1	207	TP53	7157	ENPEP	2028
CHRM3	1131	VEGFA	7422	NFKBIA	4792	SERPINE1	5054
CHRM1	1128	BCL2	596	XDH	7498	BDNF	627
ADRB1	153	CASP9	842	SOAT1	6646	HMGCR	3156
SCN5A	6331	MMP2	4313	MDM2	4193	MPO	4353
ADRA1A	148	JUN	3725	PCNA	5111	PPARA	5465
CHRM2	1129	CASP8	841	ERBB2	2064	PPARD	5467
ADRA1B	147	MMP1	4312	ICAM1	3383	Unrecognized	
GABRA1	2554	HMOX1	3162	MCL1	4170	entrezID	
TRPV1	7442	CYP3A4	1576	BIRC5	332	TEP1	7011
RXRG	6258	CYP1A2	1544	IL2	3558	TRIM26	7726
RELA	5970	ALB	213	CCNB1	891	PON1	5444
CCND1	595	CAV1	857	TYR	7299	MAP2	4133
MAPK1	5594	CTNNB1	1499	IFNG	3458	UCP2	7351
EGF	1950	MYC	4609	IL4	3565	GAP43	2596
CDK4	1019	CASP7	840	TOP2A	7153	TOP1	7150
CASP3	836	F3	2152	GSTP1	2950	CITED1	4435
PPARG	5468	GJA1	2697	XIAP	331	MMP10	4319
G6PD	2539	CCK	885	SLC2A4	6517	CRP	1401
TNFRSF1A	7132	PIK3CG	5294	INSR	3643	UCP3	7352
PRKCB	5579	NTRK2	4915	CD40LG	959	PAM	5066
PECAM1	5175	DRD1	1812	PDX1	3651	DNPEP	23549
NOS3	4846	CHRM4	1132	ADCY2	108	NUF2	83540
SCD	6319	PYY	5697	SLC2A2	6514	KCNH2	3757

contrast with non-smoker without COPD [43]. C-jun level in patients was markedly higher than that in healthy people [44].This study inferred that SZYQD may effectively prevent excessive apoptosis by inhibiting the above proteins and interfering with apoptosis, TNF, P53 signaling pathways and so on.

Vagal excitation is one of the physiological mechanisms of COPD [45]. ACh releases excessively [45], the expression of AChE is up-regulating [46], Subtypes of M receptor play their own roles and change accordingly [47–49]. Because SZYQD have effect on all subtypes of M receptor (including M1-M5), it is predicted that it may weaken the stimulation of ACh on the postsynaptic membrane of vagus nerve by inhibiting M receptor and enhancing AChE activity.

4.4. Limitations

4.4.1. Selection of drug database

When screening potential active ingredients of herbal medicine, to ensure the high oral bioavailability of drugs and possibility of drug development, OB and DL in TCMSP were initially identified as screening indicators. However, the same screening indicators don't exist when compared to data from other databases (including BATMAN-TCM, TCMID, TCMDatabase @ Taiwan, etc). After careful consideration, only the data from TCMSP is retained to ensure the results obtained under the same premise. Nevertheless, the therapeutic effect of other active ingredients on the disease in human body on other premise should not be excluded, which can be research directions of other scholars, and results can be used for reference and supplement of each other.

4.4.2. Experiment support

Generally speaking, animal or human research on the action mechanism of drugs take a lot of manpower, material resources, time, money and energy. Because of the restrictions, most of scholars can only focus on studying the changes of the level of several molecules, or the changes of levels of some key molecules in a KEGG pathway. In this study, all the data mentioned above is based on previous experimental results and computer simulations, including each active ingredient separated out from traditional Chinese herbal medicine; action targets

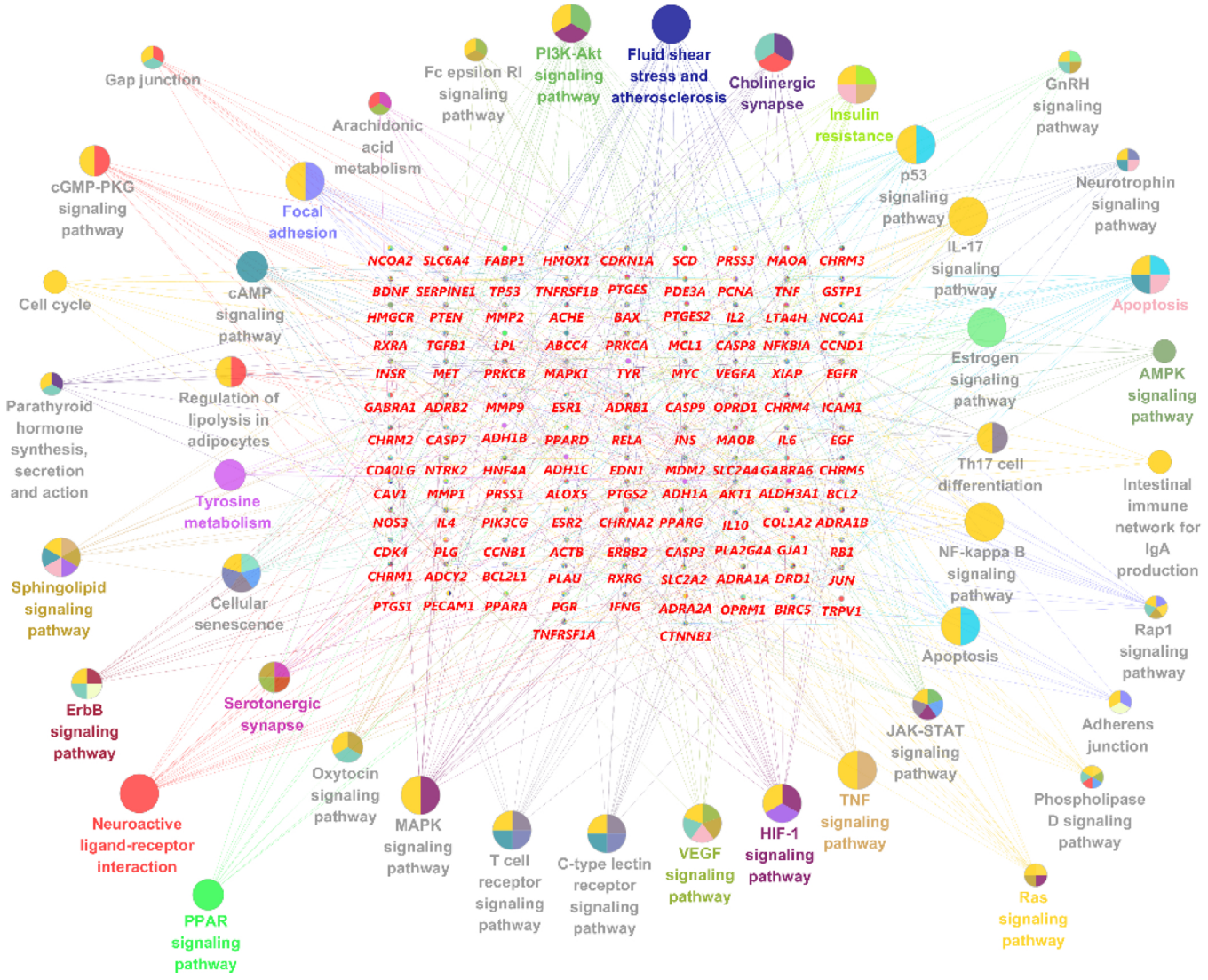

Fig. 7. Selected pathways in KEGG enrichment analysis (from Clue GO).

which have been predicted by molecular docking and confirmed by experiments; GO function, KEGG pathways, the pathogenesis of diseases, etc. By putting the data together, then we can screen in these data and identify these manually or with computers. Therefore, this study is of low cost and can obtain hundreds and thousands of action targets in the human body, as well as multiple pathways in which these action targets are located, providing a direction for subsequent research. However, the effect of SZYQD on the actual change of target level cannot be reflected in this study, this still needs to be studied or verified in detail in follow-up animal and human experiments, so as to further implement the development of new drugs and improve clinical medication thinking.

4.4.3. Inevitable updates of basic data

Due to dependence on multilevel data of multiple database, the research progress of the pathogenesis of COPD, and molecular levels change during the course of the disease. This study has the advantage of synthetically studying on large amounts of data, but there are still considerable limitations as follows.

First of all, all the data will be irregularly updated in these databases. In these databases, if any errors are found, data increases or reduces, or even the calculation method, mathematical model and inclusion criteria, which are used to characterize data, change, this study will not be able to follow those changes.

Secondly, the pathogenesis of COPD is unknown so far. This study presents that the molecular mechanism of SZYQD in the treatment of COPD is prominently in accord with the classical pathogenesis of COPD, but it does not markedly correspond with others, such as the change in the microbial groups. Moreover, if there are new findings on the pathogenesis of COPD in the future, this study will not be able to track to this new discovery.

Thirdly, because this study needs to estimate the influence of SZYQD on targets and their related pathways, so we need a preliminary assessment on whether the change of target levels in the course of the disease is in line with the influence of COPD on the final function of those pathways, and it turns out that they are basically consistent with each other after literature review. Then the basic idea is predicting that SZYQD can reverse impact on the target levels in the course of the

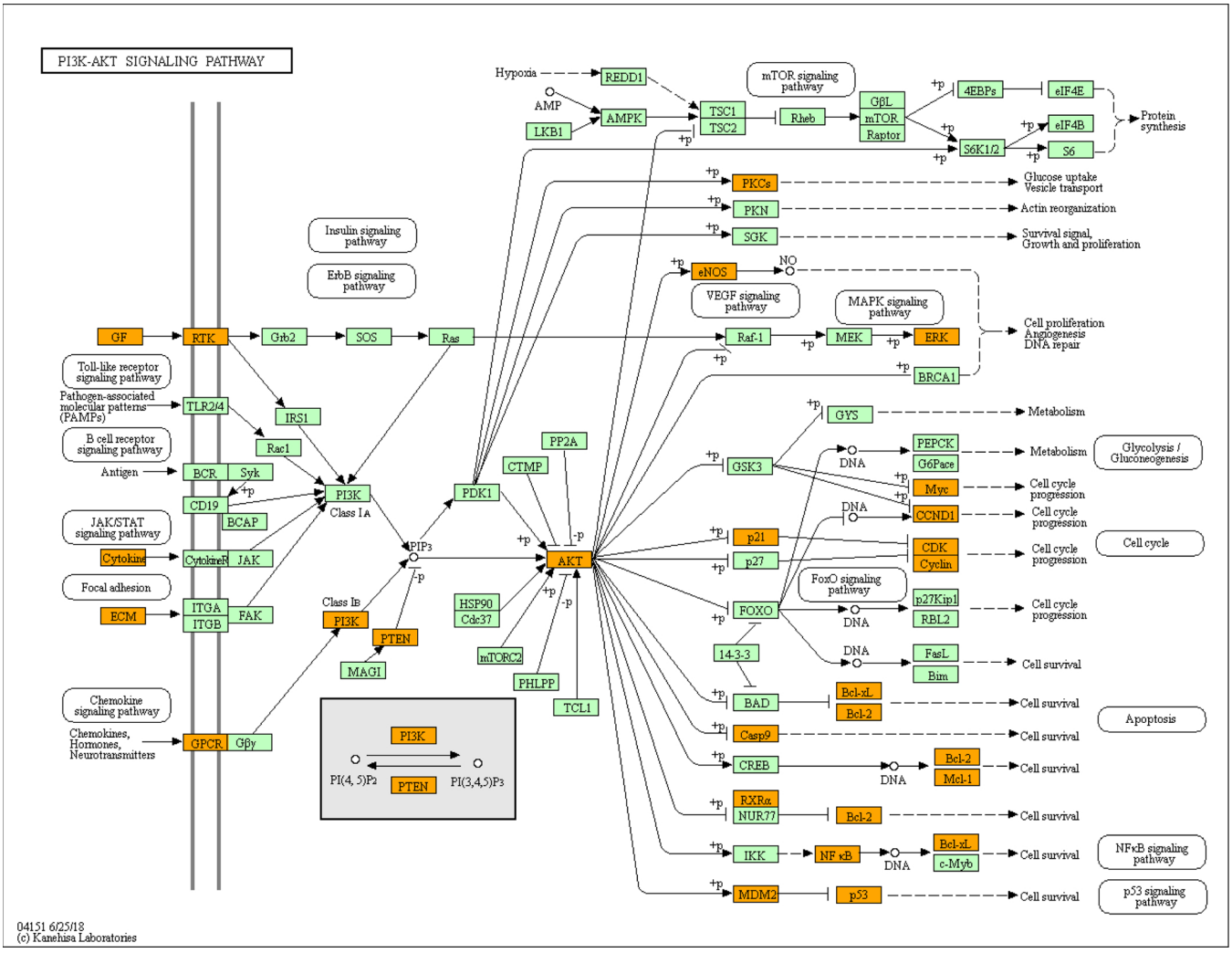

Fig. 8. PI3K-AKT signaling pathway (from KEGG PATHWAY Database).

disease, nevertheless, the actual experimental results may not be completely consistent with the prediction. In addition, various lung cells or extracellular fluid should be chosen prudently to study with in the actual experiments.

4.4.4. Research status of pharmacology

It should be noted that the active ingredients listed in Table 1 have not been decocted, because of the problem regarding the change of active ingredients after decoction is still particularly difficult to solve in this field until now. In a prescription without decoction, there are hundreds or thousands of active ingredients. Such abundant active ingredients are hard to be isolated and then identified by observing which ones have chemical reaction or not. The workload is especially huge, and it is not only one or two research team that can complete this kind of research in a short time. So until now, there are still very few herbs that have been studied in this area, even for ginseng. Only a few studies have reported several active ingredients isolated for the first time from SZYQD after decoction, because this kind of task is actually too difficult. Although this study is in accordance with all the active ingredients of SZYQD, but without data after decoction to contrast it with. It is noteworthy that the result of the molecular mechanism in this study, is still basically consistent with the pathogenesis of COPD and curative effect of SZYQD. What is more, when active ingredients of SZYQD with various modes of decoction can be isolated and identified in the future, the results of this kind of research can be complementary to others.

5. Conclusion

To sum up, SZYQD probably has the properties of being anti-oxidative, anti-inflammatory, regulating cell proliferation-apoptosis imbalance, inhibiting vagus nerve excitation and so on in the treatment of COPD by regulating cholinergic synapses, Th17 cell differentiation, Fluid shear stress and atherosclerosis, Apoptosis, PI3K-AKT, NF-κB, TNF, C-type lectin receptor and other signaling pathways. Based on the prediction of 170 targets, this study identified their interactions, GO function and KEGG pathway enrichment; and further analyzed and inferred the molecule mechanism of SZYQD, and can broaden the thinking for the subsequent pharmacological research.

Data availability

The data used to support the findings of this study are available

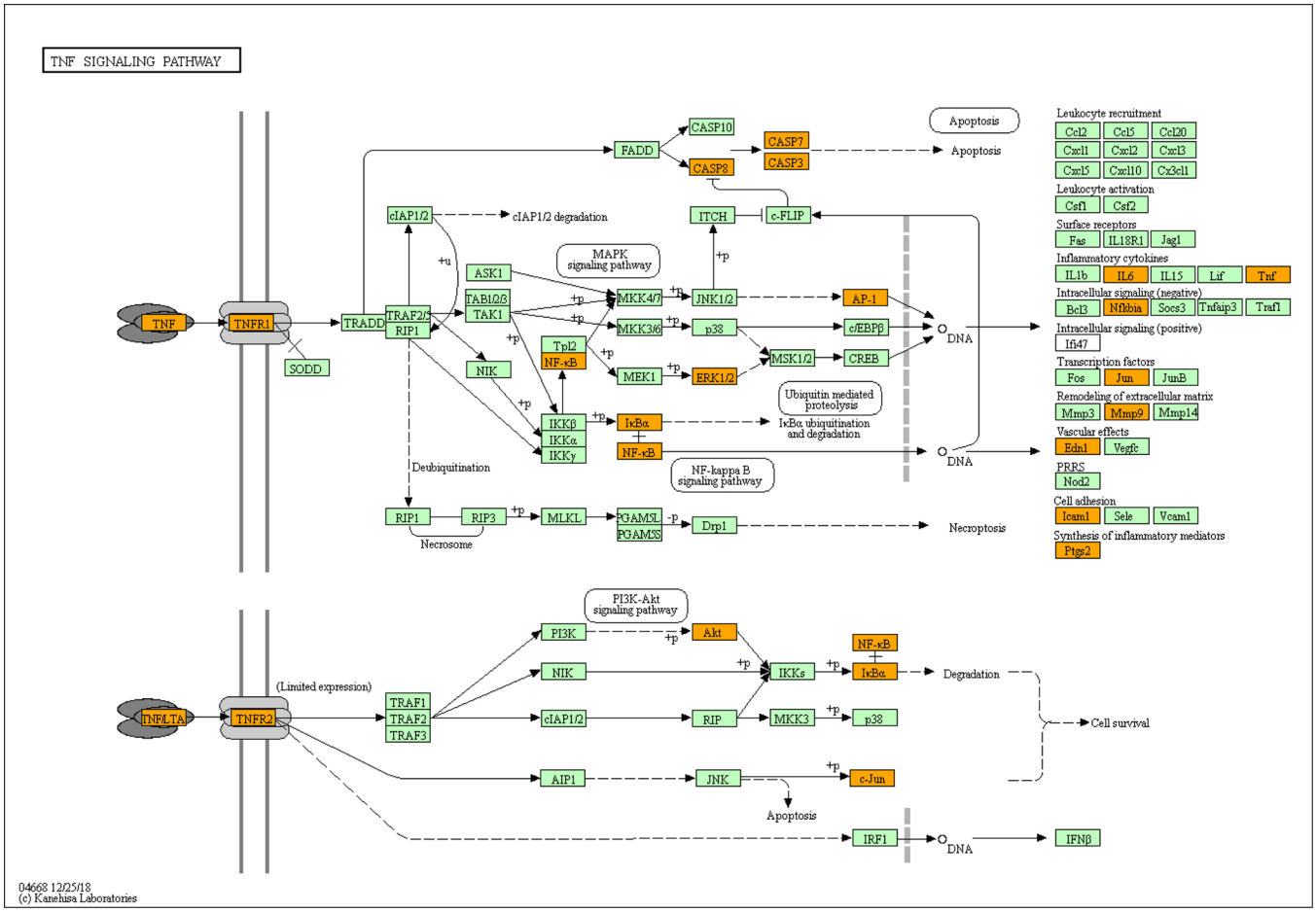

Fig. 9. TNF signaling pathway (from KEGG PATHWAY Database).

from the corresponding author upon request.

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CRediT authorship contribution statement

Fuqi Xie: Data curation, Funding acquisition, Software, Supervision, Writing - original draft, Writing - review & editing. **Wenjiang Zheng:** Data curation, Funding acquisition, Methodology, Software, Project administration. **Qian Yan:** Formal analysis, Writing - original draft. **Zijing Peng:** Supervision, Writing - original draft. **Xiaohong Liu:** Visualization.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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