Study on the Network Pharmacological Mechanism of Huanglian Wendan decoction in the treatment of Depression

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Research Article

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Abstract

Background

According to statistics, there are about 350 million patients with depression in the world, causing serious social and economic burden. Huanglian Wendan decoction has a certain clinical effect on depression. This study is based on network pharmacology and molecular docking to study the mechanism of Huanglian Wendan decoction in the treatment of depression.

Method

The active components and action targets of Huanglian Wendan decoction were obtained by searching the literature in database, and the related targets of depression were obtained. protein interaction network was constructed by STRING database, component-target network was constructed by Cytoscape3.9.0 software, enrichment analysis was carried out in KEGG database and R software package clusterprofiler, and molecular docking between active components and key antidepressant targets was carried out by AUTODOCK molecular docking software.

Result

We obtained 180 active components of Huanglian Wendan decoction and 171 targets related to depression, among which IL-6, JUN, TNF, TP53 and VEGFA may be the key targets of depression. Through the enrichment analysis of KEGG pathway, we obtained 58 signal pathways related to depression. Molecular docking showed that luteolin could form the best complex with IL-6 and TP53, beta-carotene could form the best complex with JUN and VEGFA, and kaempferol could form the best complex with TP53.

Conclusion

The antidepressant mechanism of Huanglian Wendan decoction may be related to its regulation of inflammatory factors and their activities, and has the potential to be called a complementary or alternative therapy for clinical depression.

1. Background

Depression is a mental disorder characterized by persistent and significant depression, accompanied by loss of appetite, palpitation, fear and so on. Serious people may also have mental disorders such as visual hallucinations, auditory hallucinations, delusions, and even pessimism and weariness of the world, resulting in suicidal attempts and behaviors. According to statistics, there are about 350 million patients with depression in the world, causing serious social and economic burden[1]. The prevalence rate and

recurrence rate of depression are high. It is predicted that depression may become the disease with the highest burden in China by 2030[2].

The pathogenesis of depression is still inconclusive, although commonly recognized social factors may play a role in severe depression, genomes and other biological factors also play a role.[3]

Globally, the 12-month prevalence rate of depression is 6% and about twice as likely to develop it in a lifetime. As a result, depression is a common disease in all countries, with more than 1/10 of people suffering from depression in their lives.[4] What's worse, the treatment cycle of depression is very long. If left untreated, the onset of depression usually lasts for months, sometimes even years[5]. Even if treated, it may take a year from onset to full recovery. In addition, after recovery, about half of the patients will experience a relapse of depression in their lives[6, 7].

It is worth noting that there is no significant difference in the prevalence of depression in December between high-income countries (5.5%) and low-and middle-income countries (5.9%). Therefore, depression is not caused by the modern way of life in developed countries, and at the same time, it has nothing to do with poverty.[8, 9]

In practice, depression first occurs in the middle of adolescence and lasts until the age of 40 or 50. For many people, they get sick in their twenties and the average age of onset is 25 years old[4], but a considerable number of people experience their first episode of depression before they are twenty years old.[10]

Despite the large number and heavy burden of depression, which can be traced back to half a century of extensive research and the increasing number of antidepressants, modern medicine is still far from adequate treatment of depression. there are problems such as failure to treat all symptoms, significant residual symptoms and relatively high drug resistance[11]. Therefore, it is necessary to find a new and effective drug to treat depression, More and more researchers pay attention to natural medicine[12, 13].

Huanglian Wendan decoction is a traditional Chinese medicine(TCM), which is composed of Coptis chinensis Franch, Pinellia ternata (Thunb.) Breit, Citrus reticulata Blanco, Citrus aurantium L., Poria Cocos (Schw) Wolf, Glycyrrhiza uralensis Fisch, and Zingiber officinale Roscoe. It comes from Wendan decoction in a book called "liuyintiaobian" in the Qing Dynasty. In the theory of traditional Chinese medicine, the main cause of depression is liver depression, so soothing the liver is an effective method for the treatment of depression. Huanglian Wendan decoction has the effects of clearing heat and dryness, regulating qi and resolving phlegm, and stomach and gallbladder, and has a significant effect on depression in clinic[14].

Compared with the traditional antidepressant therapy, Huanglian Wendan decoction has the advantages of broad spectrum, multi-target and low drug dependence, which is expected to replace or supplement the existing treatment methods.

Since Johns Hopkins put forward the concept of network pharmacology in 2008[15], it can describe the complex relationship among biological systems, diseases and drugs from the perspective of network, and provide a full or partial understanding of the principles of network theory and systems biology. therefore, it is considered to be the next paradigm of drug discovery. With the development of computer technology and systems biology, network pharmacology has been proved to be a feasible choice to effectively and systematically cl

arify the mechanism of traditional Chinese medicine[16, 17].

2. Material And Method

2.1 Network Pharmacology-Based Analysis

2.1.1 Identification of Candidate Components in Huanglian Wendan Decoction

All the components of all kinds of Chinese herbal medicines in Huanglian Wendan decoction were retrieved from the traditional Chinese medicine systems pharmacology (TCMSP) database(https://tcmspw.com/)[18].

2.1.2 Screening Strategy for Bioactive Components in Huanglian Wendan Decoction

Oral Chinese medicine should first consider the problem of availability, oral bioavailability (OB) is one of the important pharmacokinetic parameters. High oral bioavailability (OB ≥ 30%) is usually an important index for determining drug active substances[19].

At the same time, as a qualitative concept applied to drug design to estimate drug usefulness, DL index is also used for substance screening[20]. Those with DL index ≥ 0.18 are considered to have high drug properties.

Therefore, the compounds with OB ≥ 30% and DL ≥ 0.18 were selected as the active substances in Huanglian Wendan decoction.

2.1.3 Prediction of Drug Targets for Huanglian Wendan Decoction

Input these screened compounds into the STITSC database (https://embl.de/) and set the species as "Homo sapiens" to obtain relevant molecular information.

2.2 Depression-related Gene Acquisition and Network Construction

In the OMIM database (https://omim.org/) and the GENECARD database (https://www.genecards.org/) search interface, enter "depression, depressive, depressive disorder" to obtain depression-related genes. We screened out the parts that overlap with the active molecules of Huanglian Wendan decoction and imported them into STRING database (https://string-db.org/) to get their interaction diagram.

2.3 Gene Ontology and Pathway Enrichment Analysis for Depression-Related Targets of Huanglian Wendan Decoction

For the functional enrichment analysis of gene set, we selected the top 5 genes with degree value for KEGG analysis. We use KEGG rest API (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest KEGG Pathway gene annotation, as a background, map the gene to the background set, and use R software package clusterProfiler (version 3.14.3) for enrichment analysis to obtain the result of gene set enrichment. The minimum gene set is 5 and the maximum gene set is 5000 value of $P<0.05$ and a FDR of < 0.25 were considered statistically significant.

Meanwhile, we use the GO annotation of the genes in R software package org.Hs.eg.db (version 3.1.0) as the background, map the genes to the background set, and use R software package clusterProfiler (version 3.14.3) for enrichment analysis to obtain the results of gene set enrichment. The minimum gene set is 5 and the maximum gene set is 5000 value of $P<0.05$ and a FDR of >0.25 were considered statistically significant.

Dpression-related signaling pathways were obtained from the comparative toxicogenomics database (CTD; http://ctdbase.org/).

2.4 Construction of Networks and Analysis

In order to further study the molecular mechanism of Huanglian Wendan decoction in the treatment of depression, we used Cytoscape3.9.0 to form compound-target and target-pathway network[21]. In these graphical networks, the compounds, proteins, or pathways were expressed as nodes, whereas the compound-target or target-pathway interactions were expressed as edges.

2.5 Molecular Docking

Get the small molecule information in .mol2 format in TCMSP database (https://tcmspw.com/)), and use AUTODOCK software to add hydrogen atoms and set all rotatable bonds to flexible format and save to

.pdbqt format for molecular docking. The receptor protein data in .pdb format were obtained from the PDB database. The crystal water and small molecules in the protein structure were deleted and saved in .pdbqt format after adding hydrogen atoms in AUTODOCK software. After introducing receptor proteins and small molecules into AUTODOCK, Gridbox is established, and Gridbox wraps the active sites of the protein. Small molecules are extracted from Gridbox and exported to a .gpf file. Run the autogrid program, select the corresponding receptor proteins and small molecules to run the autodock program, then use the analysis program of AUTODOCK software to analyze the binding energy of various binding modes between small molecules and receptor proteins, derive the binding mode with the minimum binding energy as the result, and transform the results into .pdb format and then draw in the PyMol software.

3. Result

3.1 Related compounds and molecules of the Huanglian Wendan Decoction

Based on the traditional Chinese medicine systems pharmacology (TCMSP) database(https://tcmspw.com/), for Huanglian Wendan decoction (Coptis chinensis Franch, Pinellia ternata (Thunb.) Breit, Citrus reticulata Blanco, Citrus aurantium L., Poria Cocos (Schw) Wolf, Glycyrrhiza uralensis Fisch, and Zingiber officinale Roscoe), we obtain 820 related compounds. After screening OB (OB≥30%) and DL (Dl≥0.18) values, we found 180 related compounds (Table 1). After the names of these compounds were searched in the STITCH database (https://embl.de/) and only high quality (higher than 0.9) results were extracted, we obtain 3855 related molecules. After deleting the repetition, we obtained 171 related molecules (Fig1).

Table 1 Active components in Huanglian Wendan decoction

Drug	ID	compounds	OB%	DL
huanglian	MOL001454	berberine	36.86124504	0.77665
	MOL013352	Obacunone	43.28625365	0.76724
	MOL002894	berberrubine	35.73551127	0.7269
	MOL002897	epiberberine	43.09233228	0.7761
	MOL002903	(R)-Canadine	55.36687348	0.77465
	MOL002904	Berlambine	36.68090144	0.81596
	MOL002907	Corchoroside A_qt	104.9542429	0.77599
	MOL000622	Magnograndiolide	63.70888436	0.18833
	MOL000762	Palmidin A	35.35818795	0.65003
	MOL000785	palmatine	64.60111294	0.64524
	MOL000098	quercetin	46.43334812	0.27525
	MOL001458	coptisine	30.671852	0.85647
	MOL002668	Worenine	45.833181	0.86552
	MOL008647	Moupinamide	86.71215907	0.26454
chenpi	MOL000359	sitosterol	36.91390583	0.7512
	MOL004328	naringenin	59.29389773	0.21128
	MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.73643694	0.27226
	MOL005815	Citromitin	86.90404672	0.51439
	MOL005828	nobiletin	61.66943932	0.51652
zhishi	MOL013276	poncirin	36.54601263	0.74202
	MOL013277	Isosinensetin	51.15169154	0.44149
	MOL013279	5,7,4'-Trimethylapigenin	39.83272335	0.29636
	MOL013428	isosakuranetin-7-rutinoside	41.24012757	0.71616
	MOL013430	Prangenin	43.59734075	0.29428
	MOL013433	prangenin hydrate	72.63400641	0.28863
	MOL013435	poncimarin	63.62092806	0.34942
	MOL013436	isoponcimarin	63.27760017	0.31316
	MOL013437	6-Methoxy aurapten	31.23776835	0.3008

	MOL013440	citrusin B	40.79716641	0.71331
	MOL001798	neohesperidin_qt	71.16885848	0.27085
	MOL001803	Sinensetin	50.55684919	0.44634
	MOL001941	Ammidin	34.54856394	0.22355
	MOL013352	Obacunone	43.28625365	0.76724
	MOL002914	Eriodyctiol (flavanone)	41.35042713	0.2436
	MOL004328	naringenin	59.29389773	0.21128
	MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.73643694	0.27226
	MOL005828	nobiletin	61.66943932	0.51652
	MOL005849	didymin	38.55138632	0.23908
	MOL000006	luteolin	36.16262934	0.24552
	MOL007879	Tetramethoxyluteolin	43.68476462	0.37009
	MOL009053	4-[(2S,3R)-5-[(E)-3-hydroxyprop-1-enyl]-7-methoxy-3-methylol-2,3-dihydrobenzofuran-2-yl]-2-methoxy-phenol	50.75513649	0.3948
gancao	MOL001484	Inermine	75.18306038	0.53754
	MOL001792	DFV	32.76272375	0.18316
	MOL000211	Mairin	55.37707338	0.7761
	MOL002311	Glycyrol	90.77578223	0.66819
	MOL000239	Jaranol	50.82881677	0.29148
	MOL002565	Medicarpin	49.21981761	0.3351
	MOL000354	isorhamnetin	49.60437705	0.306
	MOL000359	sitosterol	36.91390583	0.7512
	MOL003656	Lupiwighteone	51.63569181	0.36739
	MOL003896	7-Methoxy-2-methyl isoflavone	42.56474148	0.19946
	MOL000392	formononetin	69.67388061	0.21202
	MOL000417	Calycosin	47.75182783	0.24278
	MOL000422	kaempferol	41.88224954	0.24066
	MOL004328	naringenin	59.29389773	0.21128
	MOL004805	(2S)-2-[4-hydroxy-3-(3-methylbut-2-	31.78703353	0.72403

	enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano[2,3-f]chromen-4-one		
MOL004806	euchrenone	30.28726099	0.57386
MOL004808	glyasperin B	65.22438608	0.43851
MOL004810	glyasperin F	75.83680013	0.53514
MOL004811	Glyasperin C	45.56380662	0.39947
MOL004814	Isotrifoliol	31.94478724	0.42422
MOL004815	(E)-1-(2,4-dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one	39.61685537	0.35077
MOL004820	kanzonols W	50.48007599	0.51704
MOL004824	(2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one	60.25040908	0.63433
MOL004827	Semilicoisoflavone B	48.77755194	0.54732
MOL004828	Glepidotin A	44.72187465	0.34685
MOL004829	Glepidotin B	64.46292386	0.34485
MOL004833	Phaseolinisoflavan	32.00810772	0.44538
MOL004835	Glypallichalcone	61.59706227	0.18993
MOL004838	8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol	58.43728091	0.38106
MOL004841	Licochalcone B	76.75735485	0.1935
MOL004848	licochalcone G	49.25496332	0.32325
MOL004849	3-(2,4-dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin	59.62247498	0.42894
MOL004855	Licoricone	63.57845938	0.4712
MOL004856	Gancaonin A	51.07519107	0.40378
MOL004857	Gancaonin B	48.79440201	0.44924
MOL004860	licorice glycoside E	32.88743479	0.27218
MOL004863	3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl)chromone	66.37125046	0.41392
MOL004864	5,7-dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)chromone	30.48877673	0.41002
MOL004866	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl)chromone	44.15196126	0.41482

MOL004879	Glycyrin	52.60657166	0.47466
MOL004882	Licocoumarone	33.21085068	0.3568
MOL004883	Licoisoflavone	41.61021885	0.41646
MOL004884	Licoisoflavone B	38.92870888	0.54714
MOL004885	licoisoflavanone	52.46624706	0.54488
MOL004891	shinpterocarpin	80.29527688	0.72746
MOL004898	(E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl)prop-2-en-1-one	46.26792256	0.3062
MOL004903	liquiritin	65.69011165	0.73893
MOL004904	licopyranocoumarin	80.36001331	0.6535
MOL004905	3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid	34.31942477	0.54718
MOL004907	Glyzaglabrin	61.06888631	0.35347
MOL004908	Glabridin	53.24514328	0.46967
MOL004910	Glabranin	52.89565508	0.31208
MOL004911	Glabrene	46.26685721	0.43902
MOL004912	Glabrone	52.51217419	0.49645
MOL004913	1,3-dihydroxy-9-methoxy-6-benzofurano[3,2-c]chromenone	48.14154235	0.42831
MOL004914	1,3-dihydroxy-8,9-dimethoxy-6-benzofurano[3,2-c]chromenone	62.90135486	0.52759
MOL004915	Eurycarpin A	43.27728425	0.37429
MOL004917	glycyroside	37.25031932	0.79156
MOL004924	(-)-Medicocarpin	40.99397199	0.95059
MOL004935	Sigmoidin-B	34.88108616	0.41455
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	71.12298901	0.18303
MOL004945	(2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)chroman-4-one	36.56537233	0.32291
MOL004948	Isoglycyrol	44.69922568	0.83845
MOL004949	Isolicoflavonol	45.16999058	0.41859
MOL004957	HMO	38.3654238	0.21067

MOL004959	1-Methoxyphaseollidin	69.98097678	0.63739
MOL004961	Quercetin der.	46.4493884	0.3343
MOL004966	3'-Hydroxy-4'-O-Methylglabridin	43.71495141	0.57406
MOL000497	licochalcone a	40.78965199	0.28517
MOL004974	3'-Methoxyglabridin	46.16150929	0.57393
MOL004978	2-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano[6,5-f]chromen-3-yl]-5-methoxyphenol	36.21429208	0.52122
MOL004980	Inflacoumarin A	39.70909598	0.32613
MOL004985	icos-5-enoic acid	30.70294255	0.19725
MOL004988	Kanzonol F	32.46833364	0.89364
MOL004989	6-prenylated eriodictyol	39.22383018	0.41259
MOL004990	7,2',4'-trihydroxy 5-methoxy-3 arylcoumarin	83.71436744	0.27136
MOL004991	7-Acetoxy-2-methylisoflavone	38.92333105	0.26217
MOL004993	8-prenylated eriodictyol	53.79476318	0.40383
MOL004996	gadelaidic acid	30.70294255	0.19725
MOL000500	Vestitol	74.65518912	0.20935
MOL005000	Gancaonin G	60.43520506	0.39404
MOL005001	Gancaonin H	50.10372327	0.78416
MOL005003	Licoagrocarpin	58.81390287	0.58498
MOL005007	Glyasperins M	72.67080984	0.59274
MOL005008	Glycyrrhiza flavonol A	41.27527733	0.59512
MOL005012	Licoagroisoflavone	57.28224098	0.48679
MOL005013	18α-hydroxyglycyrrhetic acid	41.16138694	0.7091
MOL005016	Odoratin	49.94821817	0.30487
MOL005017	Phaseol	78.76621925	0.57867
MOL005018	Xambioona	54.84916242	0.87419
MOL005020	dehydroglyasperins C	53.82326014	0.37006
MOL000098	quercetin	46.43334812	0.27525

dazao	MOL012921	stepharine	31.54786691	0.33376
	MOL012940	Spiradine A	113.5246051	0.60736
	MOL012946	zizyphus saponin I_qt	32.69113507	0.61923
	MOL012961	jujuboside A_qt	36.66570163	0.61915
	MOL012976	coumestrol	32.48702929	0.33733
	MOL012980	Daechuine S6	46.48469652	0.79151
	MOL012981	Daechuine S7	44.81774487	0.82806
	MOL012986	Jujubasaponin V_qt	36.98963109	0.63448
	MOL012989	Jujuboside C_qt	40.2624316	0.61911
	MOL012992	Mauritine D	89.12509381	0.45286
	MOL001454	berberine	36.86124504	0.77665
	MOL001522	(S)-Coclaurine	42.35064217	0.23518
	MOL000211	Mairin	55.37707338	0.7761
	MOL000449	Stigmasterol	43.82985158	0.75665
	MOL003410	Ziziphin_qt	66.9452858	0.61926
	MOL000358	beta-sitosterol	36.91390583	0.75123
	MOL004350	Ruvoside_qt	36.12101953	0.75671
	MOL000492	(+)-catechin	54.82643405	0.24164
	MOL005360	malkangunin	57.71384384	0.62642
	MOL000627	Stepholidine	33.10625074	0.54083
	MOL007213	Nuciferin	34.43102883	0.40475
	MOL000783	Protoporphyrin	30.86028979	0.55583
	MOL000787	Fumarine	59.26250458	0.82694
	MOL008034	21302-79-4	73.52245496	0.7661
	MOL008647	Moupinamide	86.71215907	0.26454
	MOL002773	beta-carotene	37.18433337	0.58358
	MOL000096	(-)-catechin	49.6763868	0.24162
	MOL000098	quercetin	46.43334812	0.27525
	MOL013357	(3S,6R,8S,9S,10R,13R,14S,17R)-17-[(1R,4R)-4-ethyl-1,5-dimethylhexyl]-10,13-	34.36766291	0.78147

		dimethyl-2,3,6,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,6-diol		
shengjiang	MOL000358	beta-sitosterol	36.91390583	0.75123
	MOL006129	6-methylgingediacetate2	48.73489111	0.32202
	MOL000449	Stigmasterol	43.82985158	0.75665
	MOL001771	poriferast-5-en-3beta-ol	36.91390583	0.75034
	MOL008698	Dihydrocapsaicin	47.07062881	0.19251
fuling	MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93214234	0.81281
	MOL000275	trametenolic acid	38.71150002	0.80199
	MOL000276	7,9(11)-dehydropachymic acid	35.105891	0.81091
	MOL000279	Cerevisterol	37.96382825	0.77061
	MOL000280	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	31.07205665	0.81528
	MOL000282	ergosta-7,22E-dien-3beta-ol	43.50708637	0.71939
	MOL000283	Ergosterol peroxide	40.36268048	0.81255
	MOL000285	(2R)-2-[(5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,4,10,13,14-pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	38.255158	0.82014
	MOL000287	3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid	38.69991401	0.8095
	MOL000289	pachymic acid	33.62791957	0.81076
	MOL000290	Poricoic acid A	30.60694619	0.76152
	MOL000291	Poricoic acid B	30.52460129	0.7463
	MOL000292	poricoic acid C	38.15135789	0.74643
	MOL000296	hederagenin	36.91390583	0.75072
	MOL000300	dehydroeburicoic acid	44.17229867	0.83458
banxia	MOL001755	24-Ethylcholest-4-en-3-one	36.08361164	0.75703

MOL002670	Cavidine	35.64183046	0.80513
MOL002714	baicalein	33.51891869	0.20888
MOL002776	Baicalin	40.12360996	0.75264
MOL000358	beta-sitosterol	36.91390583	0.75123
MOL000449	Stigmasterol	43.82985158	0.75665
MOL005030	gondoic acid	30.70294255	0.19743
MOL000519	coniferin	31.1099992	0.32308
MOL006936	10,13-eicosadienoic	39.99355408	0.20012
MOL006937	12,13-epoxy-9-hydroxynonadeca-7,10-dienoic acid	42.15217611	0.24248
MOL006957	(3S,6S)-3-(benzyl)-6-(4-hydroxybenzyl)piperazine-2,5-quinone	46.8888952	0.26989
MOL003578	Cycloartenol	38.68565906	0.78093
MOL006967	beta-D-Ribofuranoside, xanthine-9	44.71878548	0.20816

3.2 Related genes and networks of depression

Based on the OMIM database (https://omim.org/) and the GENECARD database (https://www.genecards.org/), we obtain 12861 depression-related genes. When the Huanglian Wendan decoction-related molecules were compared with depression-related molecules, 156 overlapping molecules were identified (Fig2). We import it into the STRING database (https://string-db.org/) and get the interaction diagram of these molecules (Fig3).At the same time, we also found the compounds related to these genes in Huanglian Wendan decoction (Fig4).

3.3 Functional enrichment analysis of the Huanglian Wendan decoction

After the related molecules were entered into KEGG rest API (https://www.kegg.jp/kegg/rest/keggapi.html) and R software package clusterProfiler (version 3.14.3), based on a $P < 0.05$ indicating a statistically significant singling pathway (Fig5). We use the GO annotation of genes in R package org.Hs.eg.db (version 3.1.0) as the background, map the genes to the background set, and use R package clusterProfiler (version 3.14.3) for enrichment analysis to obtain the results of gene set enrichment. Set the minimum gene set to 5 and the maximum gene set to 5000 value of $P<0.05$and a FDR of<0.25 were considered statistically significant (Fig6). A total of 98 KEGG pathways and 488 GO pathways were identified from the Huanglian Wendan decoction. Based on the CTD

database (CTD; http://ctdbase.org/), we obtained 517 related KEGG pathways of depression. 58 KEGG pathways form the Huanglian Wendan decoction were overlapped with those of depression (Fig7). The related pathways are shown in figure 5, which are Fluid shear stress and atherosclerosis Neuroactive ligand-receptor interaction Calcium signaling pathway IL-17 signaling pathway cGMP-PKG signaling pathway TNF signaling pathway Endocrine resistance, etc.

3.4 Analysis of molecular docking results

Five key antidepressant targets, such as IL6, JUN, TNF, TP53 and VEGFA, were docked with several active components acting on them by AUTODOCK software (version 4.2.6). The best conformation of docking was selected as the result of molecular docking, and the docking binding energy was extracted and sorted. The lower the binding energy is, the better the binding is. The binding energies of Luteolin with IL6 and TP53 are the lowest, the binding energies of beta-carotene with JUN and VEGFA are the lowest, and the binding energies of kaempferol and TNF are the lowest (Table 2). The best composite structure of the above active components and antidepressant targets is shown in Fig 8(Fig8).

Table2 The binding energy of proteins with their corresponding small molecules

gene	components	Binding energy
IL6	quercetin	-4.82
	luteolin	-6.69
JUN	quercetin	-3.46
	nobiletin	-3.47
	luteolin	-4.09
	formononetin	-4.27
	kaempferol	-4.05
	beta-sitosterol	-4.64
	beta-carotene	-5.1
TNF	quercetin	-3.4
	luteolin	-4.07
	kaempferol	-4.74
TP53	quercetin	-3.87
	nobiletin	-3.51
	luteolin	-4.75
	baicalein	-4.72
VEGFA	quercetin	-3.56
	luteolin	-3.72
	beta-carotene	-5.97
	baicalein	-4.36

4. Discussion

Depression is a pathological process involving a variety of neurotransmitters, brain regions and circuits, and multiple mechanisms, including the theory of monoamine transmitters, changes in the interaction between GABA and its receptors, neurobiochemical mechanisms, abnormal elevation of glutamate concentration in the brain, changes in neuropeptides and receptors in the brain, changes in cytokine levels, hippocampal neuronal nutrition / regeneration theory, astrocyte dysfunction, abnormal cellular signal mechanism, etc.[22-24]

4.1 Research on Drug targets based on Network Modeling

The characteristic of traditional Chinese medicine (TCM) decoction is that it contains a lot of active ingredients. Because of the untraceability of these ingredients, the properties of multi-components, multi-components and multi-targets of traditional Chinese medicine decoction are often confusing. Therefore, the concept of network pharmacology is put forward. In network pharmacology, the effect of multi-target drugs is often more than that of single-target drugs[25, 26].In this study, we analyzed thousands of compounds, targets and evidence-based factors in Huanglian Wendan decoction. In addition, we also analyzed the related genes and signal pathways of depression in order to find out the part of Huanglian Wendan decoction that can act on depression. The active substances in Huanglian Wendan decoction were found by constructing a network of 156 molecules interacting with depression.

Subsequently, we analyzed the compounds of each drug in Huanglian Wendan decoction [Coptis chinensis Franch, Pinellia ternata (Thunb.) Breit, Citrus reticulata Blanco, Citrus aurantium L., Poria Cocos (Schw) Wolf, Glycyrrhiza uralensis Fisch, and Zingiber officinale Roscoe] and their corresponding related genes, and constructed a network. The network model seems to show us the homogeneity and heterogeneity of each traditional Chinese medicine in Huanglian Wendan decoction. In order to better distinguish, we express the unique components of each drug separately, while the common ingredients are displayed together. Then, we get several interesting conclusions: (1) most of the ingredients in Zingiber officinale Roscoe are the same as other drugs; (2) Glycyrrhiza uralensis Fisch contains many and complex ingredients. It not only covers most of the common ingredients, but also has a lot of independent ingredients; (3) Citrus aurantium L. contains most of the ingredients in Citrus reticulata Blanco, both of which have a high degree of identity and heterogeneity. (4) Poria Cocos (Schw) Wolf does not have the same composition as other drugs, and there is a high degree of heterogeneity.

4.2 Antidepressant mechanism of main active substances

In this study, we found many active substances in Huanglian Wendan decoction which can act on depression-related targets, we selected the key and important targets, based on the existing literature, made a simple discussion on its mechanism.

4.2.1 Antidepressant effect of luteolin

In our study, we found that luteolin, the active ingredient in Huanglian Wendan decoction, has a strong effect on the key targets of depression.

Some studies support the link between inflammation, neurodegenerative diseases, oxidative stress, neuropsychological disorders (such as depression) and mild cognitive impairment[27-30]. Luteolin(3’,4’,5,7-tetrahydroxyflavone) is a common flavonoid in many fruits, vegetables, and medicinal herbs[31]. Flavonoids show many neuroprotective and inflammatory effects[32, 33].

Previous studies have shown that the oral bioavailability of flavonoids is very low[34]. However, Zhou et al drew different conclusions when studying the bioavailability of luteolin in peanut shell extract[35].

Recent studies have shown that neuroinflammation has been shown to be related to the pathogenesis of different central nervous system diseases such as depression[36].

Mariem Achour et al showed that luteolin significantly alleviated neuroinflammation by reducing the production of IL-6 by brain-derived astrocytes, decreasing the levels of serum IL-6, TNF α and corticosterone, and increasing the levels of dopamine and norepinephrine in hypothalamus. Luteolin showed the potential of astrocyte differentiation for the first time, thus highlighting its possible therapeutic role in neuroinflammatory and neurodegenerative diseases. Although the antidepressant behavioral effect of luteolin is not statistically significant, luteolin therapy regulates many signal pathways involved in the pathophysiology of depression[37].

4.2.2 Antidepressant effect of beta-carotene

In this study, we found that beta-carotene is the active ingredient second only to luteolin in Huanglian Wendan decoction. At present, the mechanism of the effect of beta-carotene on depression is not clear, but a series of social surveys and clinical studies have shown that the total intake of beta-carotene carotene may be inversely proportional to the risk of depressive symptoms[38-40].

4.2.3 Antidepressant effect of Kaempferol

In this study, we found that kaempferol is also a key active substance acting on the key target of depression in Huanglian Wendan decoction. In 1930, Professor Gyorgyti discovered a new chemical isolated from oranges. He was considered a new member of the vitamin family, initially identified as vitamin P, but later considered flavonoids[41, 42]. As mentioned above, luteolin has many neuroprotective and inflammatory effects as flavonoids, and kaempferol has similar effects as luteolin[32, 33]. Studies by Graza et al have shown that taking kaempferol and other flavonoids can show an important antidepressant regulatory effect, and the female sex of flavonoids supplementation shows sensitivity to the antidepressant imipramine, which can reverse the depression-like behavior of offspring[43]. This may be that kaempferol plays an antidepressant effect mediated by antioxidant and anti-inflammatory ability by increasing the AKT/ β-catenin cascade in the frontal cortex[44].

4.3 Shared signaling pathways of pharmacogenomics

The effect of drugs on biological cells depends more on signal pathways, and the most important thing is to determine whether the molecular nodes in the network can be identified as members of the pathway, so as to eliminate "path orphans"[45]. Fortunately, the KEGG database is constantly updating about genomes, pathways, diseases and drugs[46]. After marking the path of the network model of Huanglian

Wendan decoction, 111 shared pathways and some non-shared pathways related to depression were marked.

4.4 Inflammation and depression

Inflammatory factor is the general name of all kinds of cytokines involved in inflammatory response, which is considered to be one of the important links in the pathogenesis of depression[22]. The inflammatory process represents a series of dynamic phenomena, and the inflammatory process represents a series of dynamic phenomena, which are shown by strong vascular reactions. Neuritis is the response of central nervous system (CNS) and peripheral nervous system (PNS) to changes in dynamic balance. There are two cell systems that mediate this process: glial cells in the central nervous system and lymphocytes, monocytes and macrophages in the hematopoietic system. In 1991, Smith first proposed that depression is related to excessive secretion of inflammatory factors by macrophages[23]. Subsequently, many patients with depression were found to have abnormal genetic regulation of the immune system and immune dysfunction[47-49].In-depth studies have found that inflammatory factors can affect neurotransmitter metabolism, neuroendocrine and neuroplasticity[22-24]. Further studies have confirmed that the pathological process mediated by inflammatory factors is closely related to depression. According to the different effects, inflammatory factors can be divided into pro-inflammatory factors (such as IL-6) and anti-inflammatory factors (such as IL-13). The study found that the expression of anti-inflammatory factors decreased in the pathological process of depression, if the supplement of exogenous anti-inflammatory factors has a certain antidepressant effect, at the same time, the antidepressant effect of drugs may also be related to the up-regulated expression of anti-inflammatory factors[48, 49]. Therefore, anti-inflammatory strategies have an important prospect in the treatment of depression.

The main active components of Huanglian Wendan decoction, such as luteolin and kaempferol, can enhance the expression of anti-inflammatory factors. In the central nervous system, the active components of Huanglian Wendan decoction also show good anti-inflammatory effects. Luteolin can inhibit the activation of microglia induced by polysaccharides and the release of a variety of pro-inflammatory factors such as TNF- α[50]. Kaempferol can also effectively and safely treat depression by enhancing antioxidant capacity and anti-inflammatory effects[44]. Therefore, we believe that Huanglian Wendan decoction through the regulation of inflammatory factors and its mediation is its main mechanism for the treatment of depression.

4.5 The limitations of this study

This study is only based on network pharmacology to analyze the relevant mechanism of Huanglian Wendan decoction in the treatment of depression, and the conclusions have not been verified by experiments. follow-up studies will make further research and exploration on its related mechanism.

5. Conclusion

This is a groundbreaking study based on molecular simulation of network pharmacology to determine the mechanism of Huanglian Wendan decoction in the treatment of depression. The common signal pathway and central node as the carrier of basic biological functions make us realize why Huanglian Wendan decoction has been active in the clinical treatment of depression in traditional Chinese medicine (TCM).

At present, for the treatment of depression, drugs are mainly based on the classical monoamine transmitter theory, which has some limitations. While Huanglian Wendan decoction, as a traditional Chinese medicine, can take into account the problems of multi-target treatment and drug resistance at the same time. It has the potential to be called clinical alternative therapy for depression.

Declarations

Consent for publication

Not applicable.

Availability of data and materials

All data and materials are available at the corresponding author.

Competing interests

All authors have no conflict of interest.

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Authors' Contributions

Jun-peng Wang and Yu-jing Pan are the first authors.

( )Conception and design: Hao Chen;( )Administrative support: Hao Chen;( )Provision of study materials or patients: Yi-huang Gu; ( )Collection and assembly of data: Jun-peng Wang, Yu-jing Pan;( )Data analysis and interpretation: Jun-peng Wang ,Yu-jing Pan;( )Manuscript writing: All authors;( )Final approval of manuscript :All authors.

These authors contributed equally to this work.

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Figures

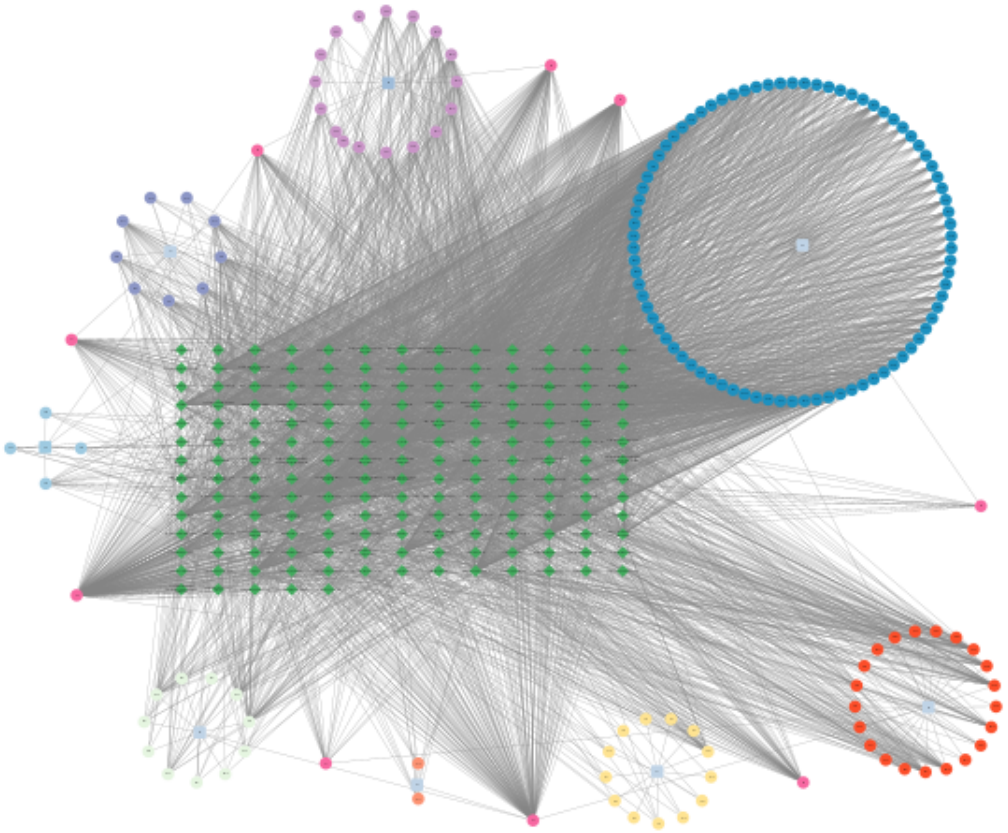

Figure 1

Active components and genes in Huanglian Wendan decoction. Among them, the purple part represents ZS, the blue part represents GC, the orange represents DZ, the yellow represents FL, the light orange represents SJ, the light green represents BX, the light blue represents CP, the dark purple represents HL, the green represents related molecules, and the others represent the common components of various traditional Chinese medicines.

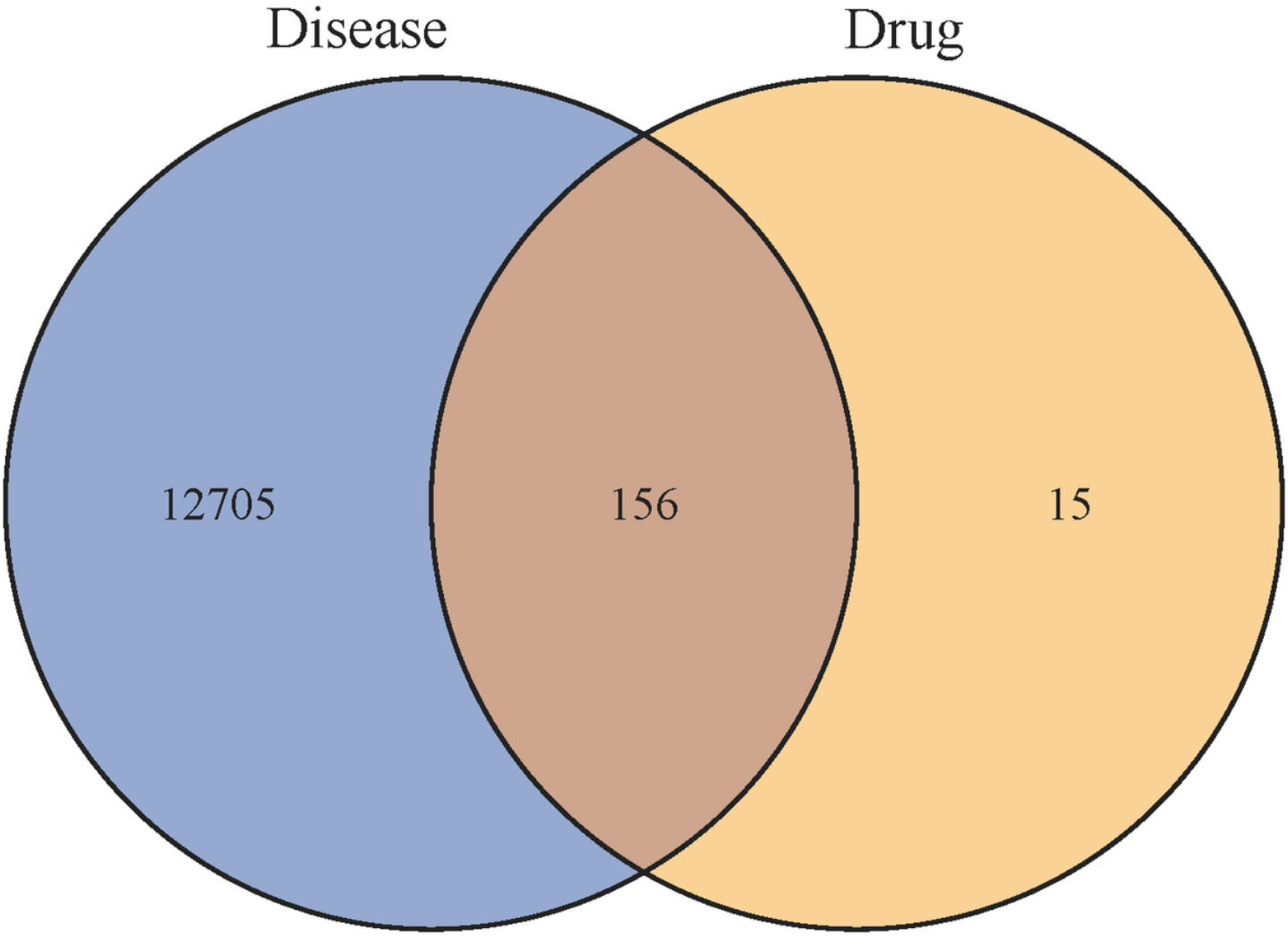

Figure 2

Common molecules of drugs and diseases

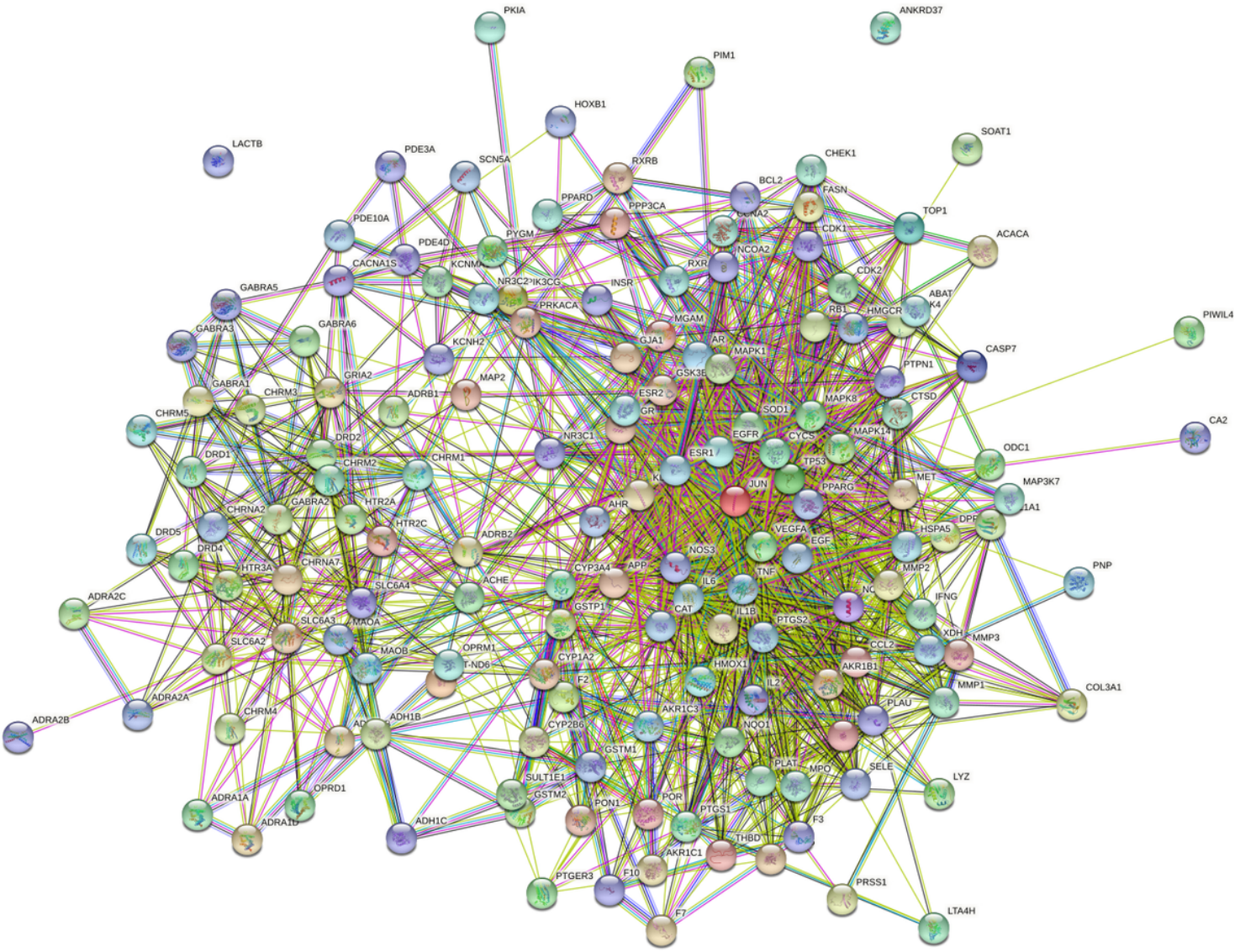

Figure 3

Relationship diagram of common targets of drugs and diseases

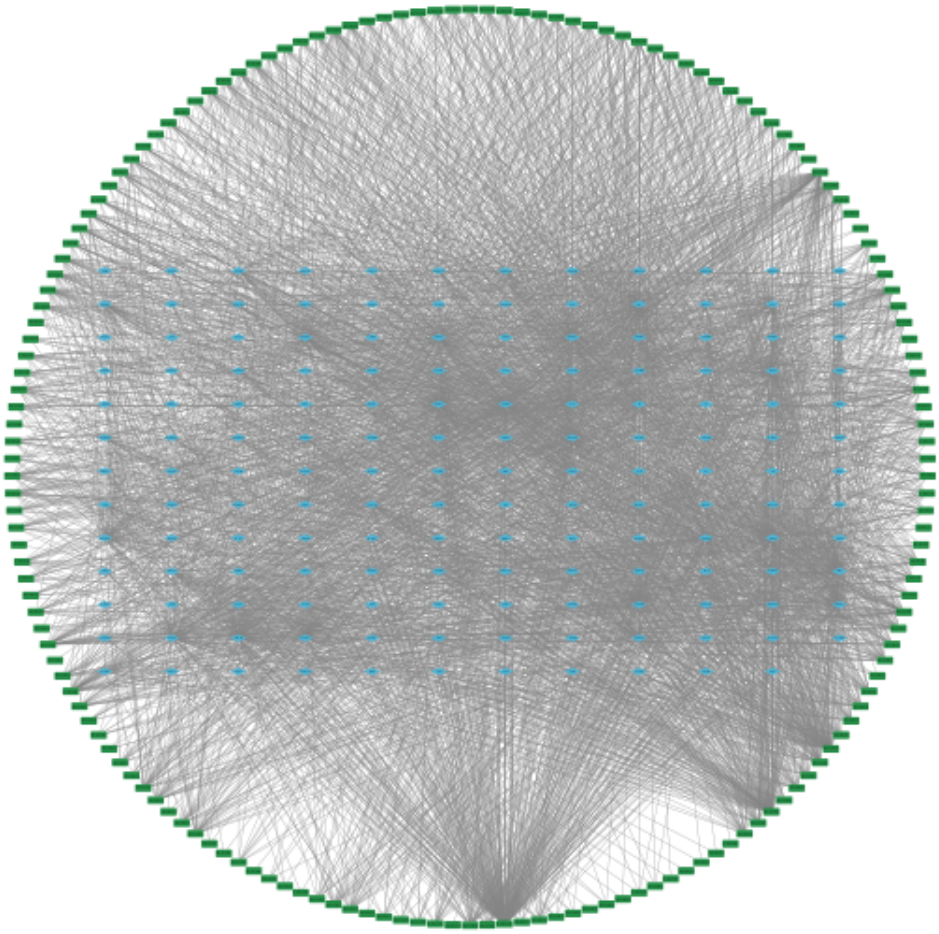

Figure 4

The common genes of drugs and diseases and the active components that affect them. Among them, the blue part represents the common molecules of disease and drug, and the green represents the active components that affect them.

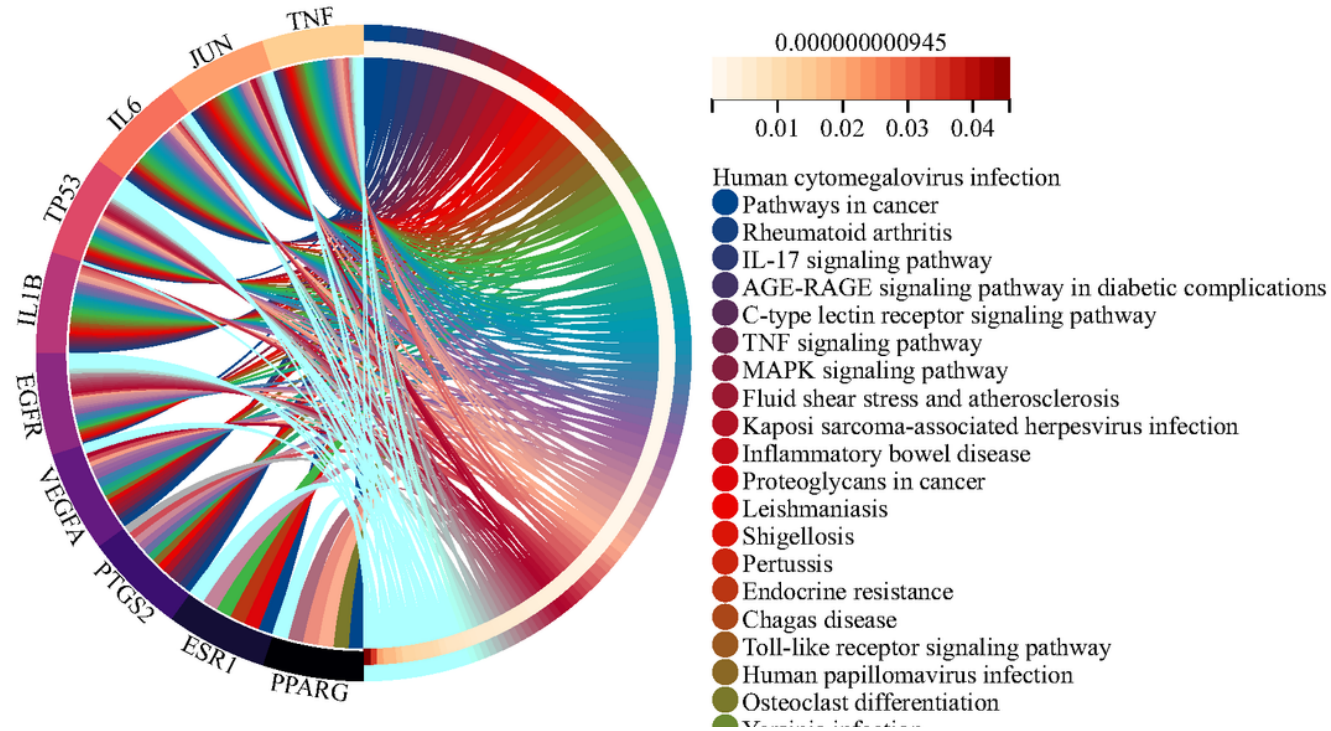

Figure 5

Circle diagram of KEGG pathway enrichment analysis

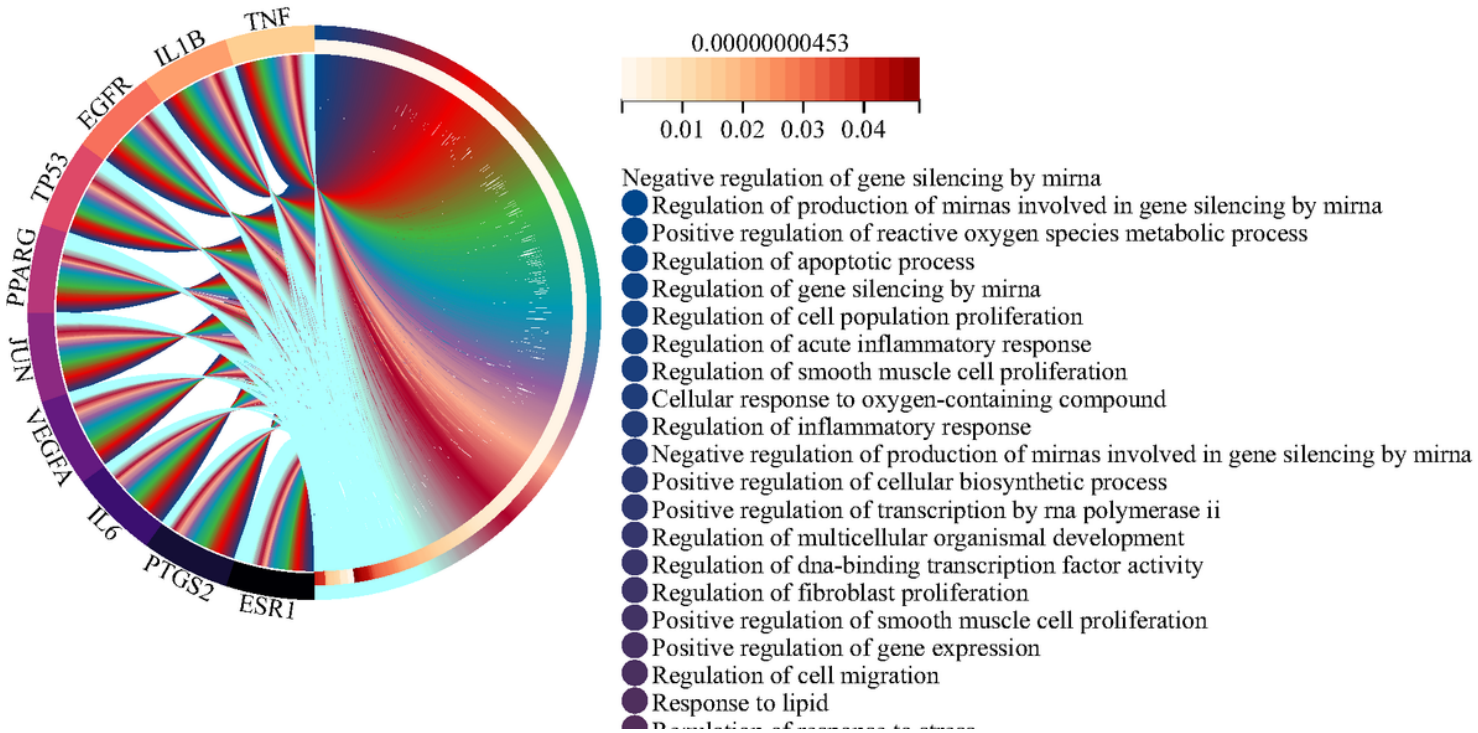

Figure 6

Circle diagram of GO enrichment analysis

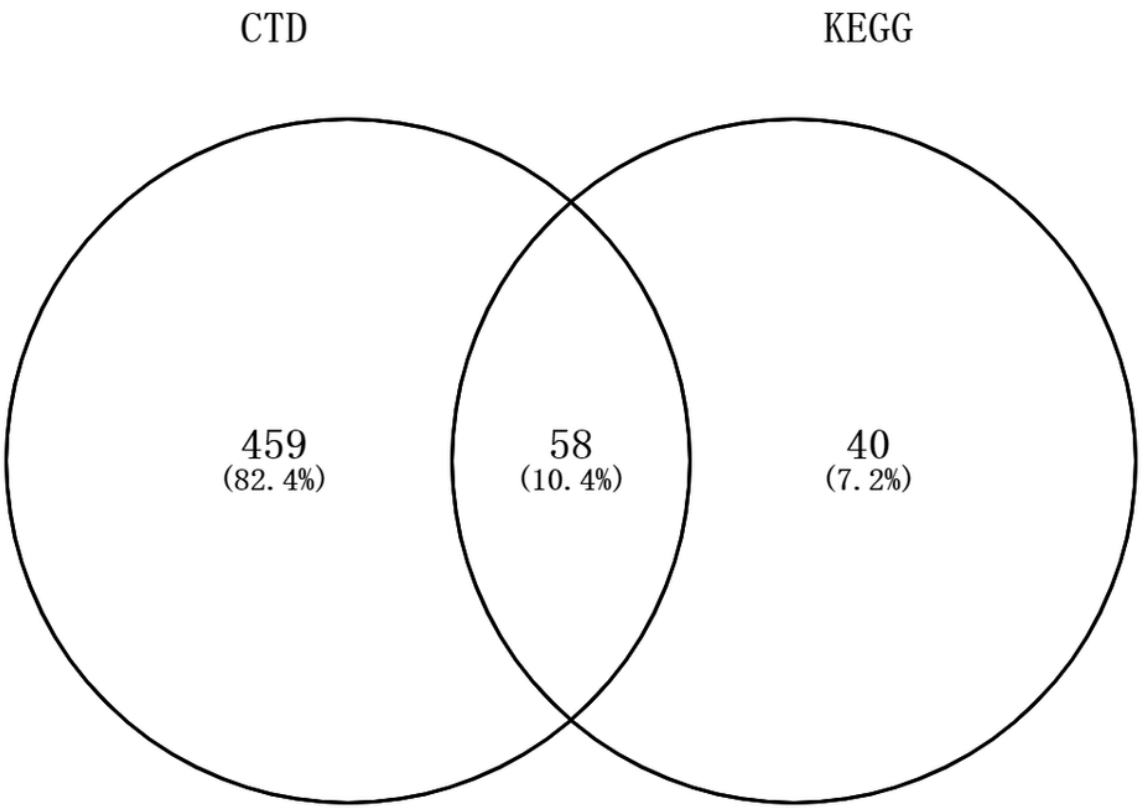

Figure 7

Common signaling pathways for diseases and drug.

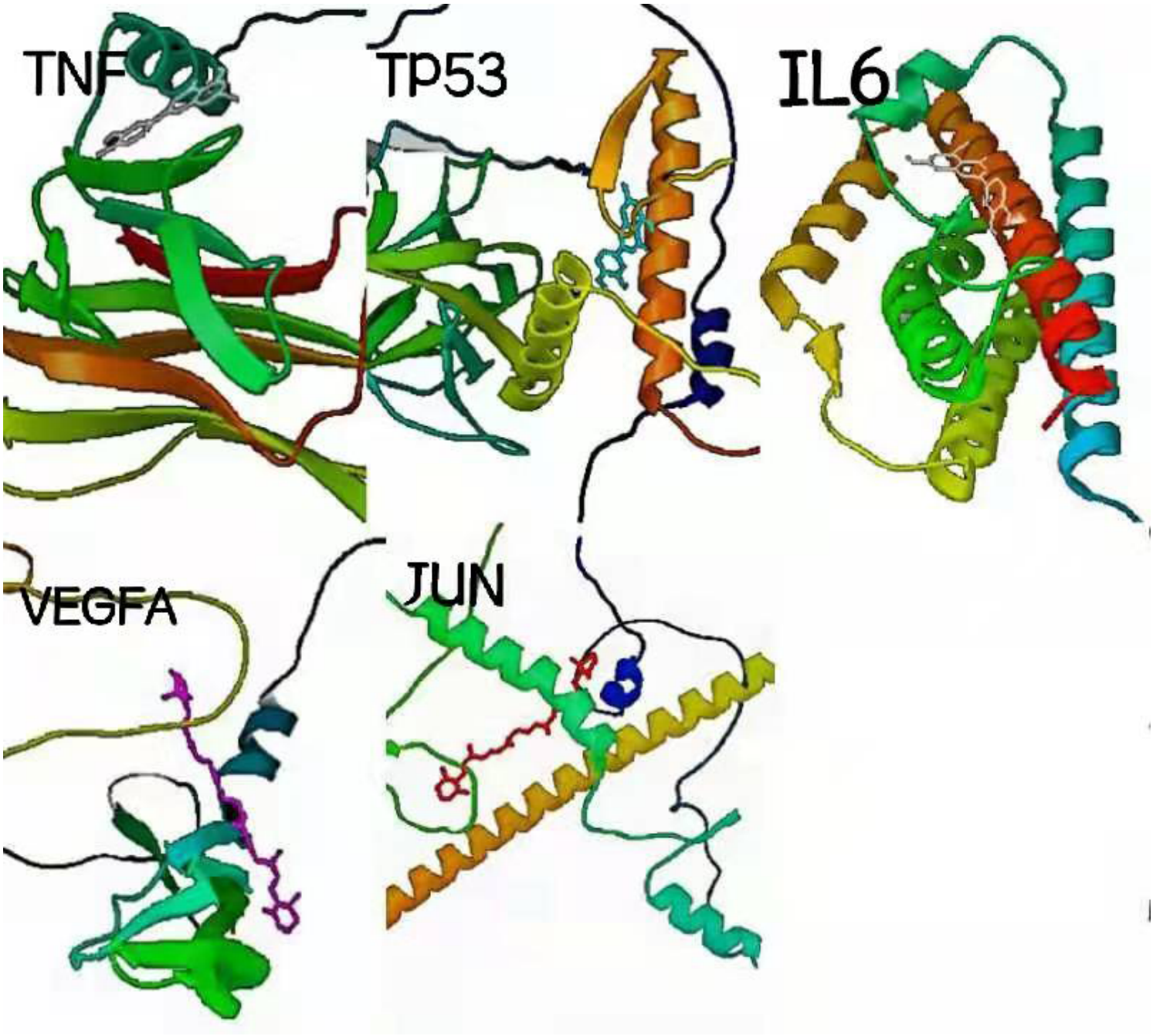

Figure 8

The target and the best complex corresponding to the best binding active componen