Molecular Mechanism of Huanglian Jiedu Decoction in Treatment of Alzheimer's Disease based on Network Pharmacology

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Abstract

Purpose: The aim of this study was to investigate the pharmacological mechanism of Huanglian Jiedu Decoction (HLJDD) in treating Alzheimer's disease through network pharmacology. HLJDD is a classic Chinese medicine prescription that is recommended in the Chinese Alzheimer's disease diagnosis and treatment guidelines. However, the mechanism of HLJDD treatment for Alzheimer's disease (AD) remains unclear because of its complicated components.

Methods: The related ingredients and targets of HLJDD in treating AD were screened by Traditional Chinese Medicine Systems Pharmacology Database and Analysis platform (TCMSP), TTD, OMIM, GeneCards, and DrugBank. The data of the protein-protein interaction (PPI) network was constructed using STRING. The Metascape database was adopted for Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and

Genomes (KEGG) enrichment analysis. AutoDockTools was used for molecular docking verification.

Results: In the treatment of AD, HLJDD demonstrated strong efficacy with its core active components including were quercetin, β-sitosterol, stigmasterol, targeting key proteins such as AKT1, TNF, and IL6. Molecular docking tests confirmed the significant binding affinity between these components and the aforementioned targets. The biological pathway of HLJDD in treating AD primarily involves the modulation of IL-17, TNF, and other inflammatory cytokines to regulate their impact on nerve functions.

Conclusions: HLJDD may treat AD by inhibiting neuroinflammation through a comprehensive, multi-component, multi-target, and multi-pathway method.

Key Words

Huanglian Jiedu Decoction; Alzheimer's disease; Network pharmacology; Molecular docking; Target prediction

Introduction

Alzheimer’s disease (AD) is the most common type of senile dementia and is characterized by an insidious and progressive degenerative process of the central nervous system. Memory dysfunction, thinking, and behavioral issues are the primary clinical features. The pathogenesis of AD is highly complex and remains inconclusive, with mainstream hypotheses including the Aβ cascade reaction, Tau protein abnormality hypothesis, cholinergic hypothesis, neuroinflammation, metabolic disorders, and vascular injury [1]. Currently, FDA-approved

clinical drugs for AD primarily consist of acetylcholinesterase inhibitors (AchE) and N-methyl-D-aspartate (NMDA) receptor antagonists. However, these drugs target a single factor, exhibit low drug resistance, and often lead to adverse effects, resulting in unsatisfactory clinical outcomes for AD treatment. Chinese medicine compound therapy has the advantages of multiple targets, multiple components and multiple pathways of action. Therefore, traditional Chinese medicine possesses unique benefits for the treatment of AD.

Huanglian Jiedu decoction (HLJDD) was initially documented in *The Handbook of Prescriptions for Emergencies.* It consists of four herbs: Coptidis rhizome (Huanglian or HL), Scutellariae radix (Huangqin or HQ), Phellodendri chinensis cortex (Huangbo or HB), and Gardeniae fructus (Zhizi or ZZ) in Chinese. HL serves as the sovereign medical, while HQ and HB, the minister medicinals, are responsible for clearing the heat of triple energizers. ZZ acts as both assistant medicinal and courier medicinal, aiding in clearing the heat of triple energizers and directing heat downward. Several studies have revealed the primary active components of HLJDD, including cognitive improvement [2], anti-inflammatory [3], anti-endotoxin [4], anti-atherosclerosis [5], and other effects. HLJDD is recognized as a classical heat-clearing medicinal agent and is widely employed in treating moderate and severe dementia because of its beneficial effects.

Network pharmacology is a comprehensive discipline that integrates the theories of systems biology, pharmacology, information networks and computer science. Using computer simulation and various databases, network pharmacology screens drug molecular targets and

disease-related targets. By employing network visualization and network analysis systems, it sheds light on the complex network connections among drugs, targets, and diseases. It constructs a "drug-target" system to analyze and predict the action mechanism of drugs at multiple levels. The systematic and holistic features of network pharmacology align with the principle of holistic thinking emphasized by traditional Chinese medicine [6].

This study employs network pharmacological methods to determine the molecular mechanism of HLJDD in treating AD, because of its complex composition and multi-level effects. HLJDD shows multi-target effects in addressing AD, however the specific active components and mechanism of action remain unclear. The goal is to establish a theoretical foundation for future investigations. The workflow of the study is illustrated in **Figure 1**.

Materials and Methods

Collection and screening the active components of HLJDD

The components and targets of the four herbs in HLJDD were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php) [7]. Following TCMSP recommendations, the components were screened based on 2 thresholds of ADME: Oral bioavailability (OB) ≥30% and Drug-likeness (DL) ≥0.18. At the same time, the published literature was reviewed and organized to supplement the known targets of the undetected active components. The Uniprot database (https://www.uniprot.org/uniprot/) was used to translate the targets of the active components into gene symbols.

Searching for potential AD-related targets of HLJDD

We conducted a search using "Alzheimer's disease" as keyword to identify *Homo sapiens* genes associated with Alzheimer's disease . AD-related targets for the treatment of AD were extracted from the TTD (https://db.idrblab.net/ttd/) [8], OMIM (http://www.omim.org/), GeneCards (https://www.genecards.org/) and DrugBank (https://go.drugbank.com/) databases. After consolidating all targets and removing duplicate entries, we compiled a list of targets related to AD by Venny (https://bioinfogp.cnb.csic.es/tools/venny/).

Constructing the protein-protein interaction (PPI) network

We constructed the PPI by the STRING database (https://string-db.org) [9]. The biological species selected was *Homo sapiens (human)*, with the minimum interaction threshold set to "highest confidence" (>0.9).

Topological analysis

Cytoscape3.9.1 was employed to build the network diagram encompassing the components,targets and pathways. Subsequently, CytoHubba, an embedded tool in Cytoscape, was used to analyze the network topology parameters of potential components. Screening was performed by Degree Centrality (DC) to determine the hub genes.

Gene Ontology (GO) Functional Annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis

Input potential targets into Metascape, setting the threshold at P＜0.01 and selecting *Homo sapiens* as the biological species for both GO enrichment analysis and KEGG pathway analysis. Saved the data results and visualized them using bubble charts, mapped with micro-biosis (https://cn.bing.com).

Molecular Docking Validation

Molecular docking is a method used to evaluate the ability of an active drug ingredient to bind to a target. The PDB database (https://www.rcsb.org/) was used to obtain the structural formulae of small molecules in PDB format. The docking of receptor proteins and small ligand molecules was performed using AutoDockTools, and the docking results were visualized through Pymol.

Results

1. Active components of HLJDD

A total of 58 chemical components were identified through TCMSP active components screening, meeting the criteria of ADME (OB≥30%, DL≥0.18). Specifically, these components encompassed 11 species of HL, 28 species of HQ, 24 species of HB, and 9 species of ZZ. Notably, there were overlaps in the components, with 4 types common to both HL and HB, 1 shared among HL, HB, and ZZ, 2 present in both HL and HQ, and 2 shared across HQ, HB, and ZZ (**Table 1**). The analysis further revealed 128 targets for HL, 72 targets for HQ, 122 targets for HB, and 120 targets for ZZ. Upon consolidation and elimination of duplicates, a total of 138 unique targets were identified.

2. Prediction of potential anti-AD targets of HLJDD

A total of 2462 Alzheimer's disease (AD)-related targets were obtained by combining data from the GeneCards database (targets with a score > 10) with information from the OMIM, TTD, and DrugBank databases. Duplicate targets were removed through a merging process. Subsequently, 137 overlapping targets were identified as potential targets for Huli Jingdan Decoction (HLJDD) in AD treatment. Consequently, a final set of 137 potential targets for HLJDD in the context of AD treatment was established (**Fig. 2**).

3. PPI network construction

The potential targets were analyzed using STRING to construct the PPI network (**Fig. 3**). The network contained 137 nodes and 337 edges, with an average node degree of 4.92 and an average local clustering coefficient of 0.511. Therefore, it can be seen from Figure.2 that the targets of HLJDD are closely related to each other in the treatment of AD, indicating that HLJDD may exert its efficacy by acting on multiple targets.

4. The "Components-Targets-Pathways" network diagram construction

CytoScape 3.9.1 was used to construct the network diagram of the "Components-Targets-Pathways" (**Fig. 4**), in which the top 10 active components (ranked by degree) were quercetin (MOL000098, A1), β-sitosterol (MOL000358, B1), stigmasterol (MOL000449, B2), kaempferol (MOL000422, ZZ1), wogonin (MOL000173, HQ2), isocorypalmine (MOL000790, HB2), palmatine (MOL000785, A3), (S)-Canadine

(MOL001455, HB4), (R)-Canadine (MOL002903, HL1), coptisine (MOL001458, A5). CytoHubba was then used for network topology analysis, with darker node colors indicating higher DC and more interaction. The results revealed top 10 core targets in AD treatment with HLJDD: TP53, AKT1, TNF, IL6, MAPK1, IL1B, ESR1, IFNG, FN1 andCCND1 (**Fig. 5**), with TP53 having a high DC of 29 (**Table 2**).

5. GO enrichment and KEGG Pathway analysis

The top 20 results obtained from the analysis using Metascape for the treatment of AD with HLJDD included the enrichment of the GO **(Fig. 6)** and KEGG pathways (**Fig. 7)**, respectively. The analysis revealed significant involvement in potential biological processes such as response to oxidative stress and reactive oxygen species. Moreover, molecular functions were identified, including neurotransmitter receptor activity and postsynaptic neurotransmitter receptor activity. The cellular components linked to these processes were found to be synapses, dendrites, and neuronal cell bodies. Additionally, the enriched pathways encompassed IL-17 signaling pathways and neurodegenerative pathways. Detailed results of the target pathway enrichment can be found in **Table 3**, providing a comprehensive overview of the findings.

6. Molecular docking validation

To analyze the top 5 active components and core targets, molecular docking was conducted using AutoDockTools 1.5.7 software. In this process, a lower binding energy indicates a more effective docking interaction between the component and the target. The docking results

revealed that all binding energies were less than or equal to -4 KJ/mol, demonstrating superior binding between the components and targets (**Table 4**). The binding energies of the first 5 interactions were visualized using PyMol 2.2.0 software, as shown in (**Fig. 8**).

Discussion

AD, as the most common form of dementia and a prominent neurodegenerative disease requiring attention, has imposed significant burdens on patients, their families, and society at large. The disease's pathological mechanism is intricate, with previous studies highlighting the beneficial role of Chinese medicine compound prescriptions in its treatment [10, 11]. HLJDD, a renowned classic prescription, is known for its efficacy in clearing heat, detoxifying, and facilitating fire purging, specifically targeting dementia arising from phlegm, stasis, and heat. Baicalin can reduce neuroinflammation and oxidative stress in rats with Alzheimer's disease by inhibiting microglial activation. It also regulates the levels of neurotransmitters such as methionine and glutamine, correcting imbalances and addressing energy metabolism disordersl [12, 13]. This was demonstrated by changes in peripheral system biomarkers SOD, GSH-Px, AMPK, SIRT-1, and MDA, indicating that HLJDD can improve cognitive function. Moreover, a 70% ethanol extract from gardenia can boost serum catalase activity, lower acetylcholinesterase levels, and improve rats' spatial learning and memory [14]. These findings underscore the potential neuroprotective effects of HLJDD and its medicinal components in combating AD and enhancing cognitive function across various biological pathways.

In this study, the significant role of HLJDD in the treatment of AD was investigated through the employment of network pharmacology to screen its active components, which include quercetin, β-sitosterol, and stigmasterol. Quercetin, a compound found in HLJDD, has been shown to inhibit the neuroinflammatory process by downregulating pro-inflammatory cytokines. Additionally, quercetin has the ability to stimulate neuronal regeneration, exerting its neuroprotective role by antagonizing cytotoxicity induced by oxidative stress in neurons [15]. Beta-sitosterol has been demonstrated to decrease the deposition of Aβ in the brains of AD mice and partially restore synaptic function [16]. Furthermore, AMPK triggers the NF-κB and NLRP3 pathways. Stigmasterol inhibits the microglial inflammation response to Aβ42 oligomers and lessen neuroinflammation in AD mice [17]. Molecular docking results also suggested that active components such as quercetin, β-sitosterol, and stigmasterol exhibit strong binding with core targets, indicating their potential therapeutic efficacy in the treatment of AD within the context of HLJDD.

In this study, AKT1, TNF, and IL-6 were identified as the primary targets for AD treatment. AKT, a protein kinase, plays a crucial role in various biological processes and pathologies such as cell growth, proliferation, and neurodegenerative diseases [18]. Among the isoforms of AKT, AKT1 is highlighted, and elevated levels of AKT1 phosphorylation have been associated with Aβ deposition and Tau protopathy, impacting neuronal and synaptic damage [19]. Neuroinflammation, characterized by elevated levels of proinflammatory cytokines in the cerebrospinal fluid of AD patients, is closely linked to disease progression. TNF, comprising TNF-α and TNF-β subtypes, along with IL-6, contributes to this inflammatory

response. Upon stimulation by Aβ, microglia are overactivated, leading to the release of pro-inflammatory cytokines like TNF-α and IL-6, exacerbating neuroinflammation and neurotoxicity in AD progression [20]. Pathway enrichment analysis revealed that HLJDD may influence AD treatment through its impact on IL-17, TNF, and other signaling pathways. The IL-17 signaling pathway, which promotes an inflammatory response, has been found to be associated with AD progression, affecting Aβ deposition, tau protein phosphorylation, and blood-brain barrier integrity in the early stages of AD. The involvement of IL-17 in cognitive dysfunction and neuroinflammation has been established [21, 22]. TNF, as a key mediator of neuroinflammation, can activate either neuroprotective or neurodegenerative cell responses through its receptors TNFR1 and TNFR2 [23]. Thus, HLJDD may have a therapeutic impact on AD by regulating IL-17, TNF, and other signaling pathways.

This study utilized network pharmacology and molecular docking as research methods to investigate the potential active components, targets, and pathways of HLJDD for treating AD. The research findings indicated that individual compounds in HLJDD could target multiple proteins, and the same protein could be involved in various signaling pathways. These results suggest that the therapeutic effects of HLJDD on AD may stem from the synergistic interactions among its multiple components, targets, and pathways. By integrating network pharmacology and molecular docking approaches, this study lays the groundwork for the clinical application of HLJDD in AD treatment, providing a preliminary scientific rationale. Moreover, it presents a promising avenue for further elucidating the specific mechanisms of HLJDD in treating AD and for exploring novel clinical strategies for managing the disease.

Key Points:

1. Huanglian Jiedu Decoction can alleviate Alzheimer's disease's symptoms.
2. Huanglian Jiedu Decoction can alleviate the pathological damage caused by Alzheimer's disease through multiple ways and multiple targets.
3. It is necessary to further verify the mechanism of Huanglian Jiedu Decoction in treating Alzheimer's disease.

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DISCLOSURES:

The authors have nothing to disclose.

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Table 1. The active components of HLJDD.

NO	MOL ID	Molecule Name	OB（%）	DL
A1	MOL000098	quercetin	46.43	0.28
A2	MOL000622	magnograndiolide	63.71	0.19
A3	MOL000785	palmatine	64.6	0.65
A4	MOL001454	berberine	36.86	0.78
A5	MOL001458	coptisine	30.67	0.86
A6	MOL002668	worenine	45.83	0.87
A7	MOL002894	berberrubine	35.74	0.73
A8	MOL002897	epiberberine	43.09	0.78
HL1	MOL002903	(R)-Canadine	55.37	0.77
HL2	MOL002904	berlambine	36.68	0.82
HL3	MOL002907	corchoroside A_qt	104.95	0.78
HQ1	MOL000073	ent-Epicatechin	48.96	0.24
HQ2	MOL000173	wogonin	30.68	0.23
HQ3	MOL000228	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	55.23	0.2
B1	MOL000358	β-sitosterol	36.91	0.75
B2	MOL000449	stigmasterol	43.83	0.76
HQ4	MOL000359	sitosterol	36.91	0.75
HQ5	MOL000525	norwogonin	39.4	0.21
HQ6	MOL000552	5,2'-Dihydroxy-6,7,8-trimethoxyflavone	31.71	0.35
HQ7	MOL001689	acacetin	34.97	0.24
HQ8	MOL002714	baicalein	33.52	0.21
HQ9	MOL002879	diop	43.59	0.39
HQ10	MOL002909	5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone	33.82	0.45
HQ11	MOL002914	eriodyctiol (flavanone)	41.35	0.24
HQ12	MOL002915	salvigenin	49.07	0.33
HQ13	MOL002917	5,2',6'-Trihydroxy-7,8-dimethoxyflavone	45.05	0.33
HQ14	MOL002925	5,7,2',6'-Tetrahydroxyflavone	37.01	0.24
HQ15	MOL002927	skullcapflavone II	69.51	0.44
HQ16	MOL002928	oroxylin a	41.37	0.23
HQ17	MOL002932	panicolin	76.26	0.29
HQ18	MOL002933	5,7,4'-Trihydroxy-8-methoxyflavone	36.56	0.27
HQ19	MOL002934	neobaicalein	104.34	0.44
HQ20	MOL002937	dihydrooroxylin	66.06	0.23
HQ21	MOL008206	moslosooflavone	44.09	0.25

HQ22	MOL012245	5,7,4'-trihydroxy-6-methoxyflavanone	36.63	0.27
HQ23	MOL012246	5,7,4'-trihydroxy-8-methoxyflavanone	74.24	0.26
HQ24	MOL012266	rivularin	37.94	0.37
HB1	MOL000787	fumarine	59.26	23.46
HB2	MOL000790	isocorypalmine	35.77	2.47
HB3	MOL001131	phellamurin_qt	56.6	14.89
HB4	MOL001455	(S)-Canadine	53.83	6.56
HB5	MOL001771	poriferast-5-en-3β-ol	36.91	5.07
HB6	MOL002641	phellavin_qt	35.86	15.81
HB7	MOL002643	delta 7-stigmastenol	37.42	5.27
HB8	MOL002644	phellopterin	40.19	-1.64
HB9	MOL002651	dehydrotanshinone II A	43.76	23.71
HB10	MOL002662	rutaecarpine	40.3	8.21
HB11	MOL002663	skimmianin	40.14	-2.43
HB12	MOL002666	chelerythrine	34.18	6.32
HB13	MOL002670	cavidine	35.64	5.78
HB14	MOL005438	campesterol	37.58	4.83
HB15	MOL006422	thalifendine	44.41	5.99
ZZ1	MOL000422	kaempferol	41.88	0.24
ZZ2	MOL001406	crocetin	35.3	0.26
ZZ3	MOL001941	ammidin	34.55	0.22
ZZ4	MOL003095	5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl) chromone	51.96	0.41
ZZ5	MOL004561	sudan III	84.07	0.59
ZZ6	MOL007245	3-Methylkempferol	60.16	0.26

Herb

HL, HB, ZZ
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HL, HB
HL, HQ
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Table 2. Top 10 core targets in AD treatment with HLJDD.

NO	Hub Gene	Degree
1	TP53	29
2	AKT1	27
3	TNF	23
4	IL6	19
5	MAPK1	16
6	IL1B	15
7	ESR1	15
8	IFNG	14
9	FN1	13
10	CCND1	12

Table 3. Enrichment results of AD target pat

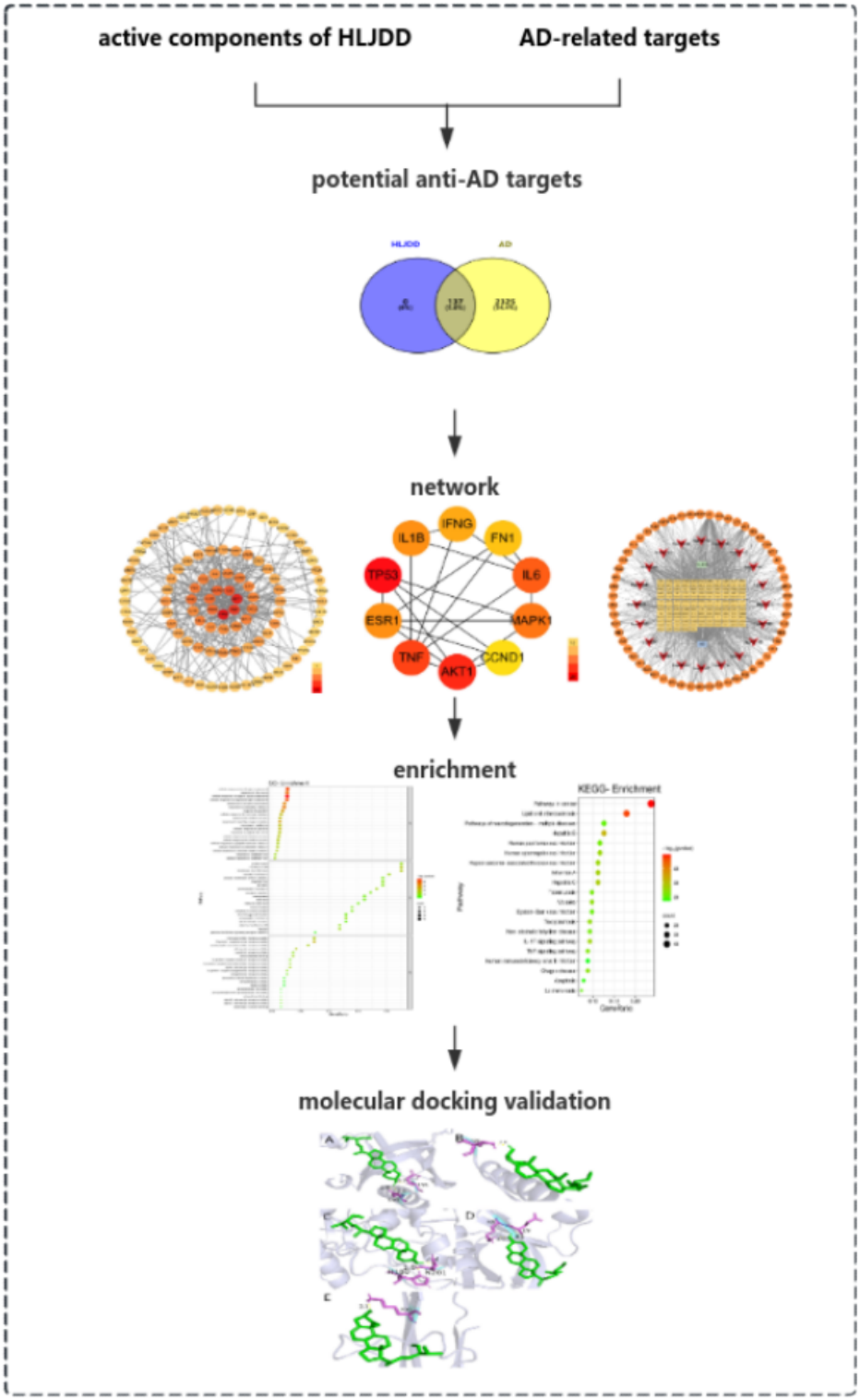
GO	Description	Count
hsa05200	Pathways in cancer	49
hsa05417	Lipid and atherosclerosis	37
hsa05161	Hepatitis B	26
hsa05160	Hepatitis C	23
hsa05164	Influenza A	23
hsa04657	IL-17 signaling pathway	19
hsa05167	Kaposi sarcoma-associated herpesvirus infection	23
hsa05163	Human cytomegalovirus infection	24
hsa05145	Toxoplasmosis	19
hsa05162	Measles	20
hsa05142	Chagas disease	18
hsa04668	TNF signaling pathway	18
hsa05152	Tuberculosis	20
hsa05165	Human papillomavirus infection	24
hsa04932	Non-alcoholic fatty liver disease	19
hsa05169	Epstein-Barr virus infection	20
hsa05140	Leishmaniasis	15
hsa05022	Pathways of neurodegeneration - multiple diseases	26
hsa04210	Apoptosis	16
hsa05170	Human immunodeficiency virus 1 infection	18

:hway regulated by HLJDD.

LogP
-49.95292154
-47.56139879
-32.32697146
-27.61024615
-26.71596954
-25.66472621
-25.40864786
-25.38720219
-24.10338032
-23.84925454
-23.17567175
-22.24865244
-21.52248748
-21.34905467
-21.28826547
-20.50271665
-20.05713349
-20.02463744
-17.67616364
-17.26562441

Table 4. Docking results of core components and key target molecules.

Target \ Component	quercetin	β-sitosterol	stigmasterol	kaempferol	wogonin
TP53	-5.49	-6.78	-7.7	-5.43	-5.56
AKT1	-5.46	-7.31	-7.79	-6.13	-4.89
TNF	-5.52	-6.7	-7.24	-6.81	-5.5
IL6	-5.28	-6.88	-7.1	-5.74	-5.48
MAPK1	-6.45	-6.89	-7.32	-6.18	-5.99


active components of HLJDD
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CCND1
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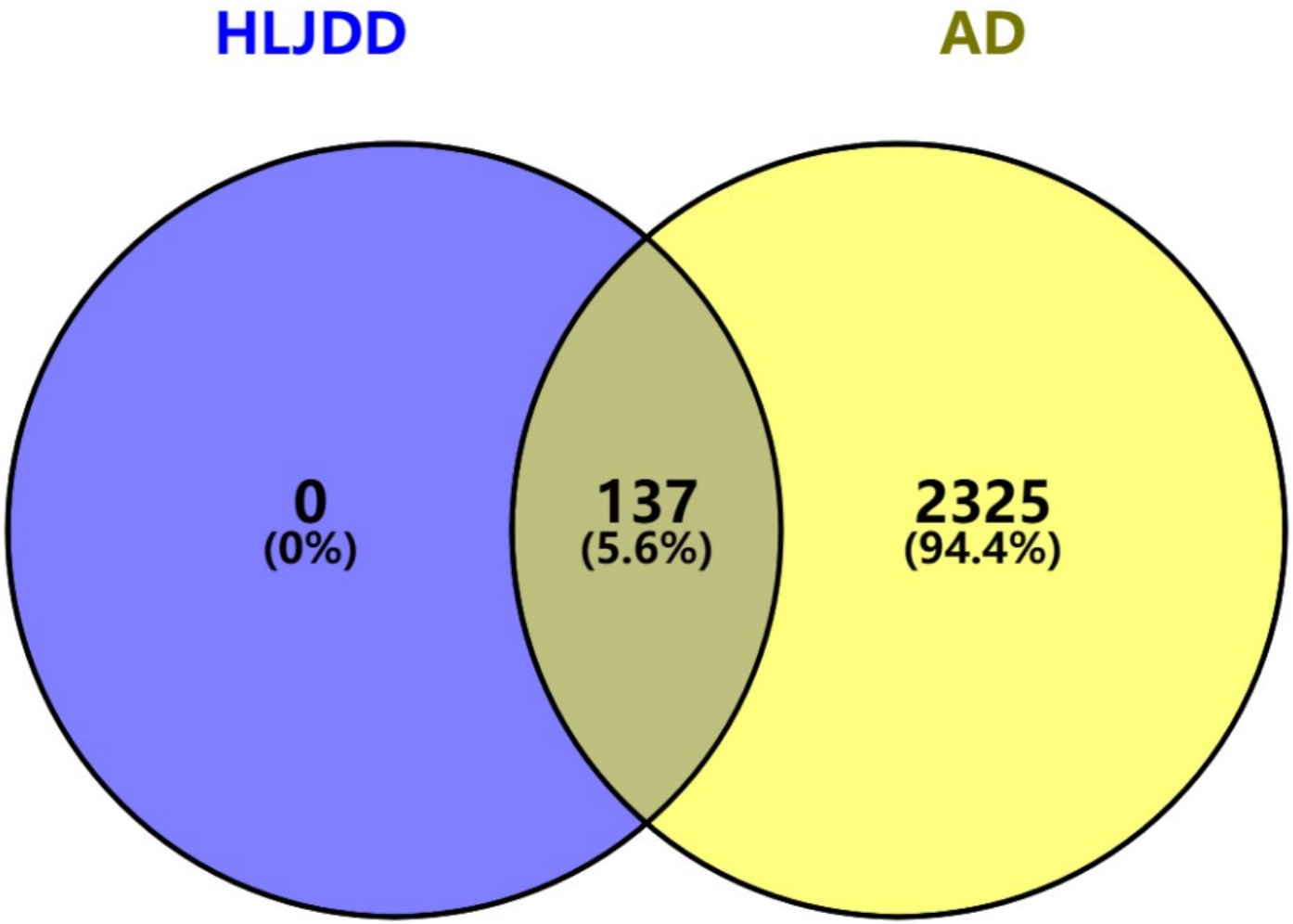
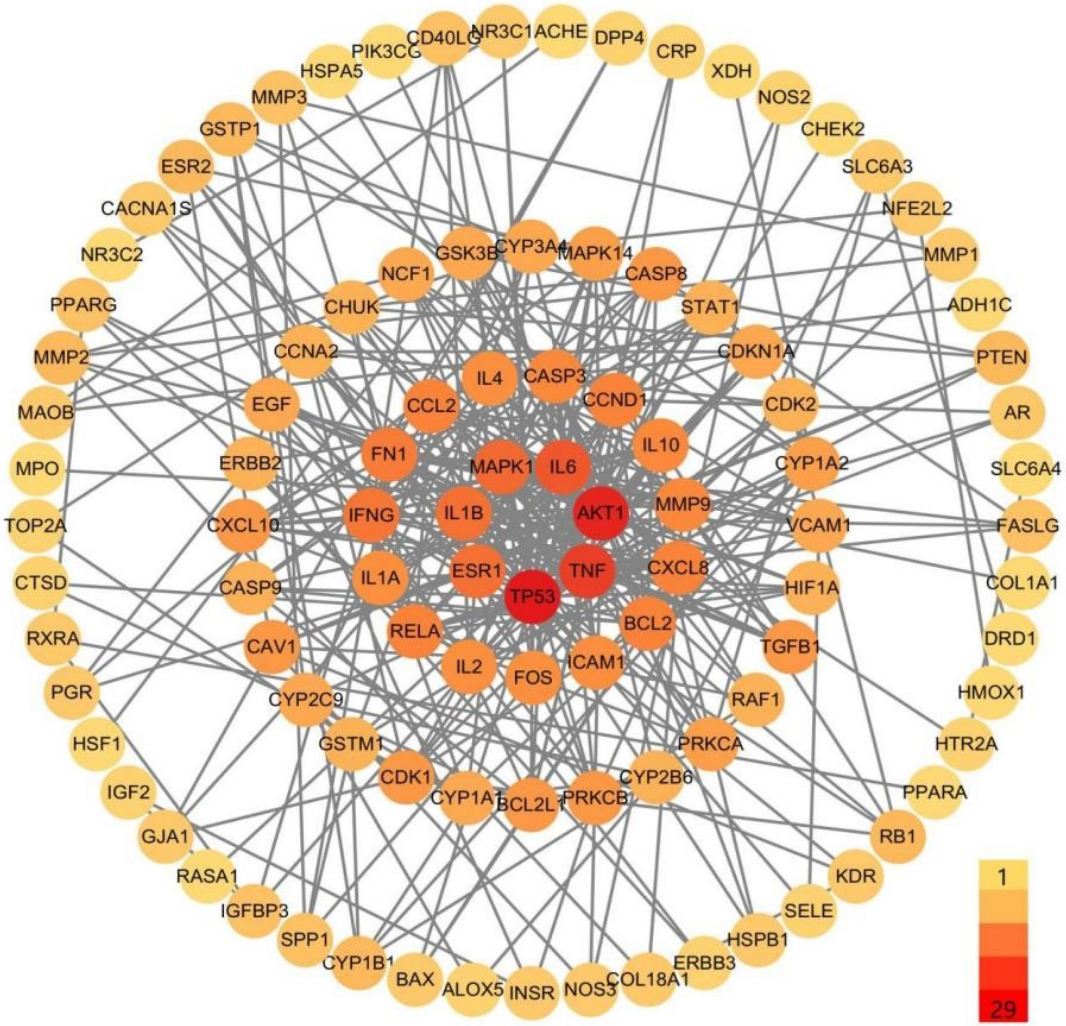
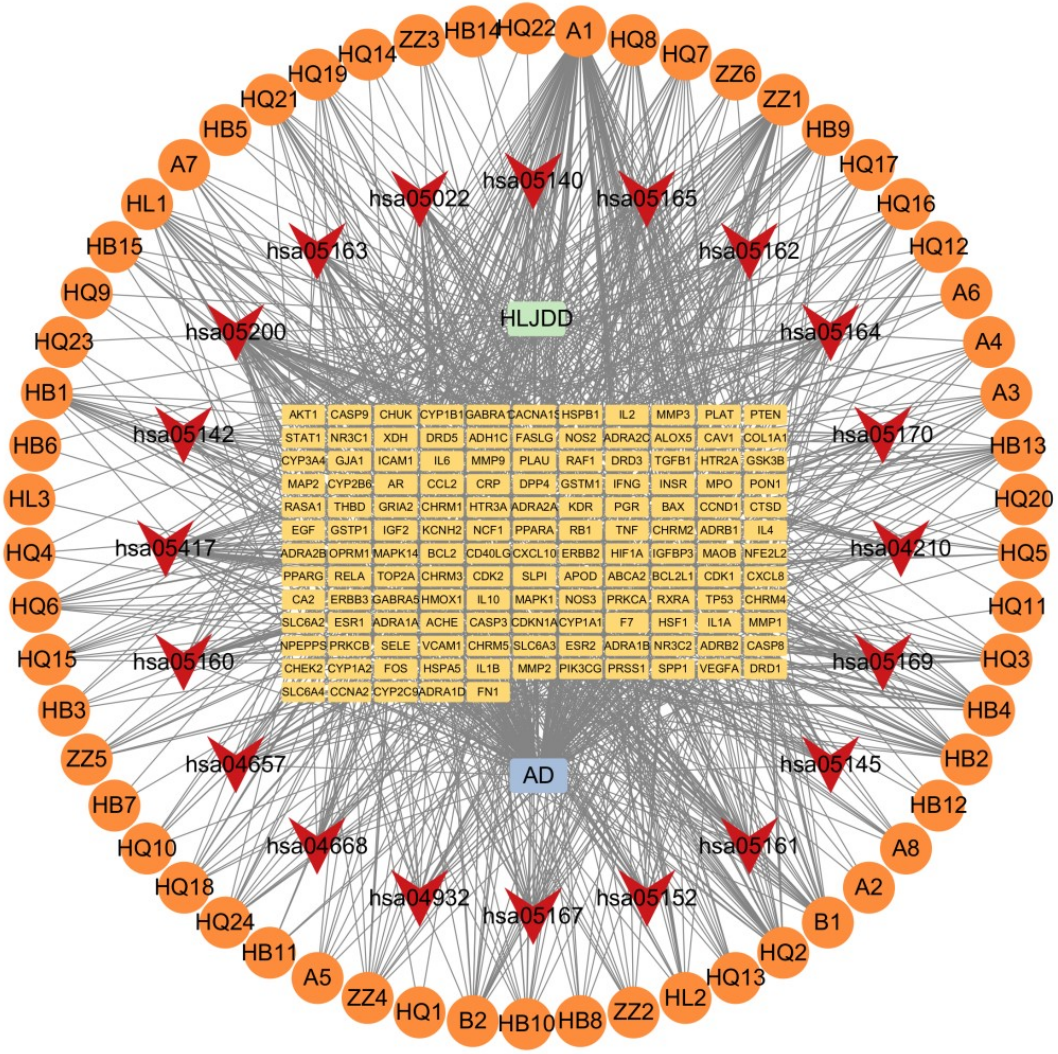
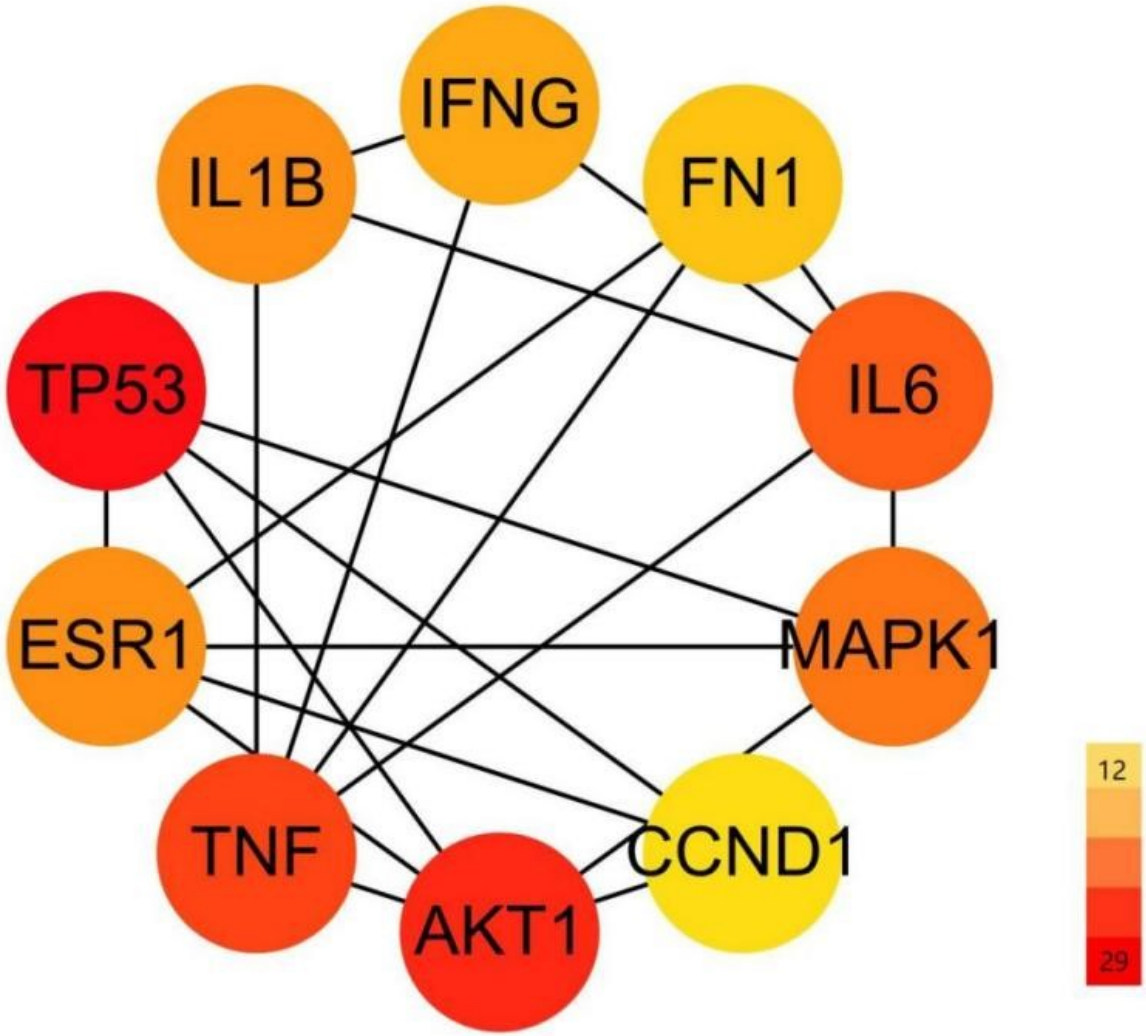
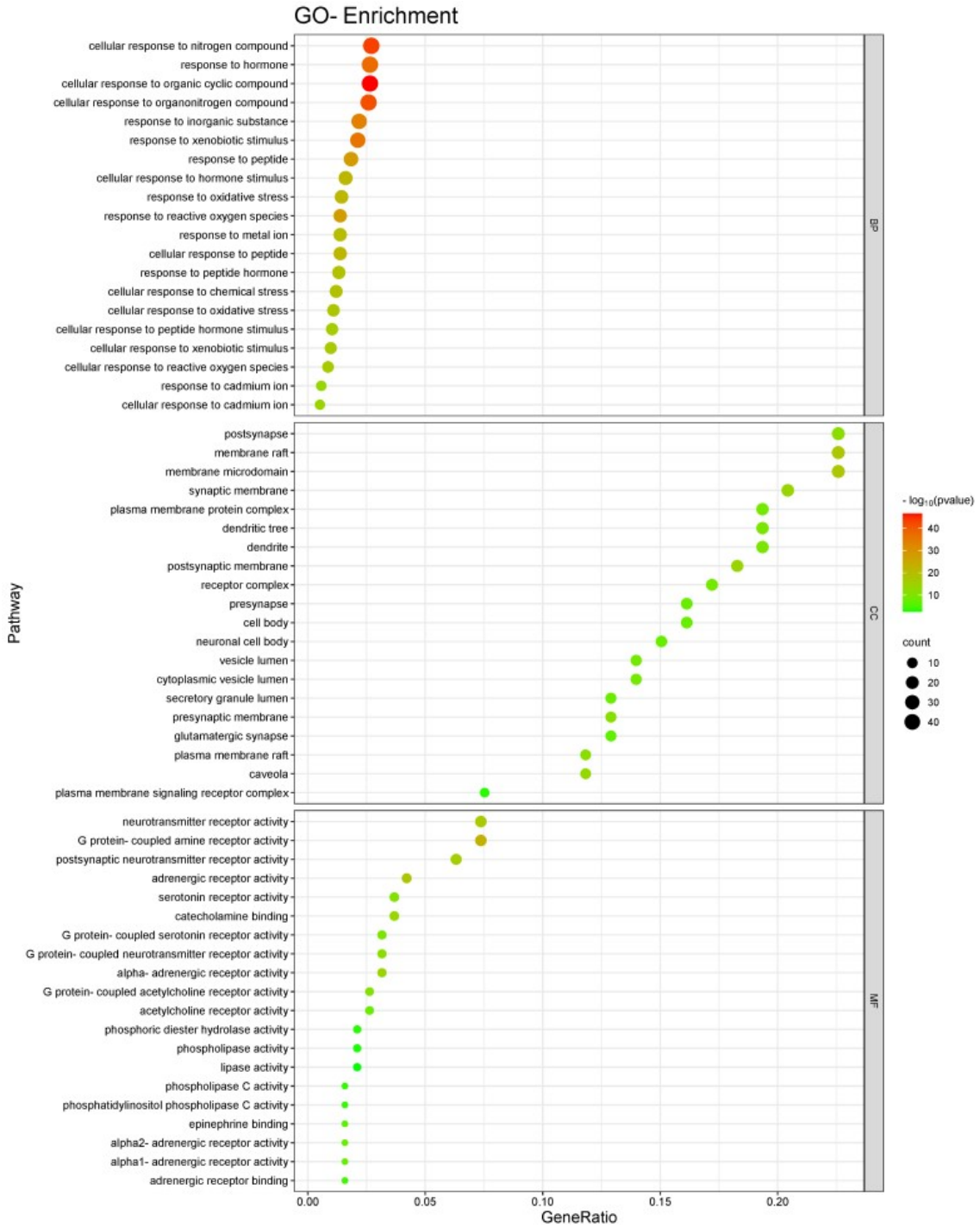
molecular docking validation

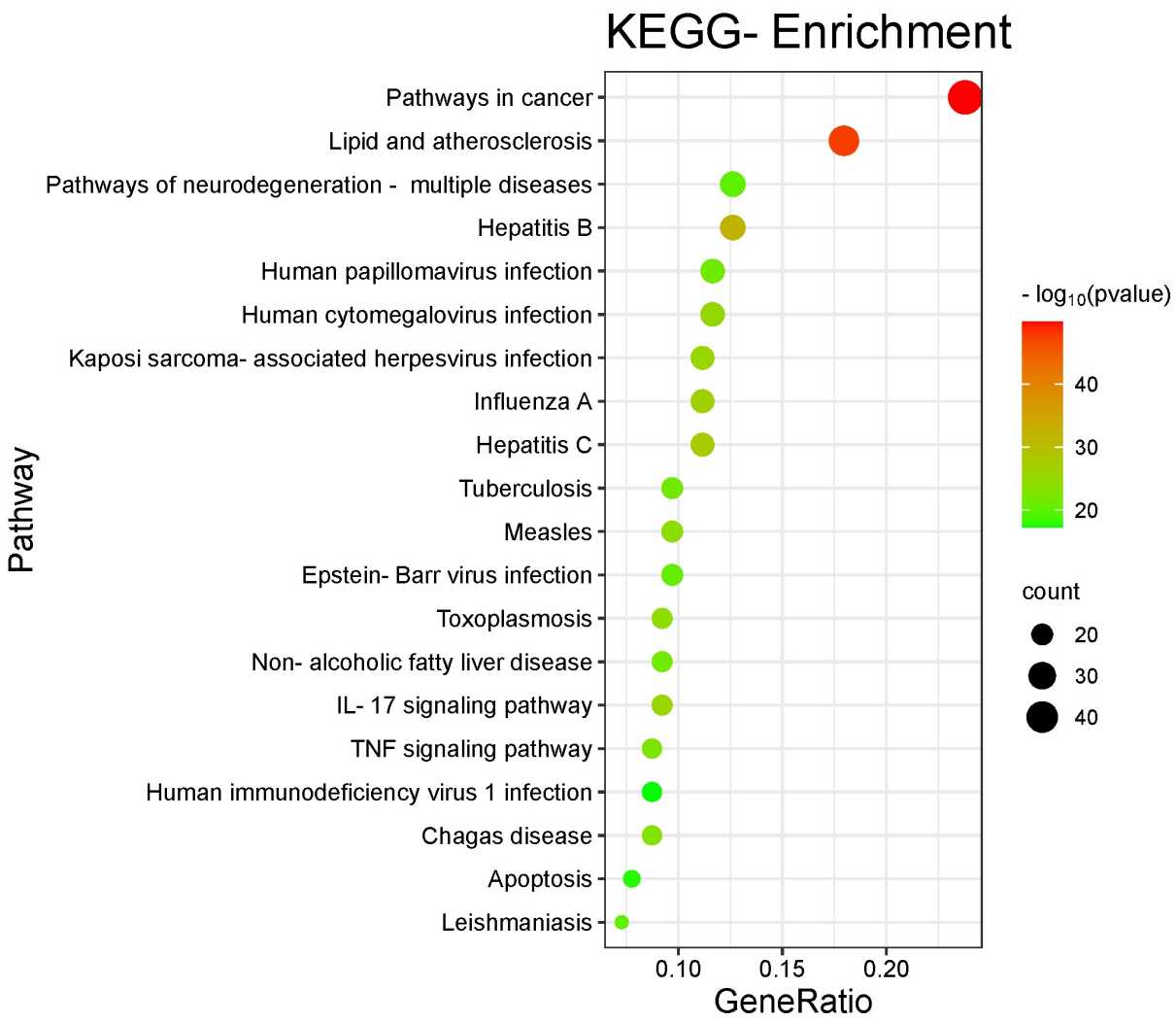

HLJDD
AD
0
(0%)
137
(5.6%)
2325
(94.4%)

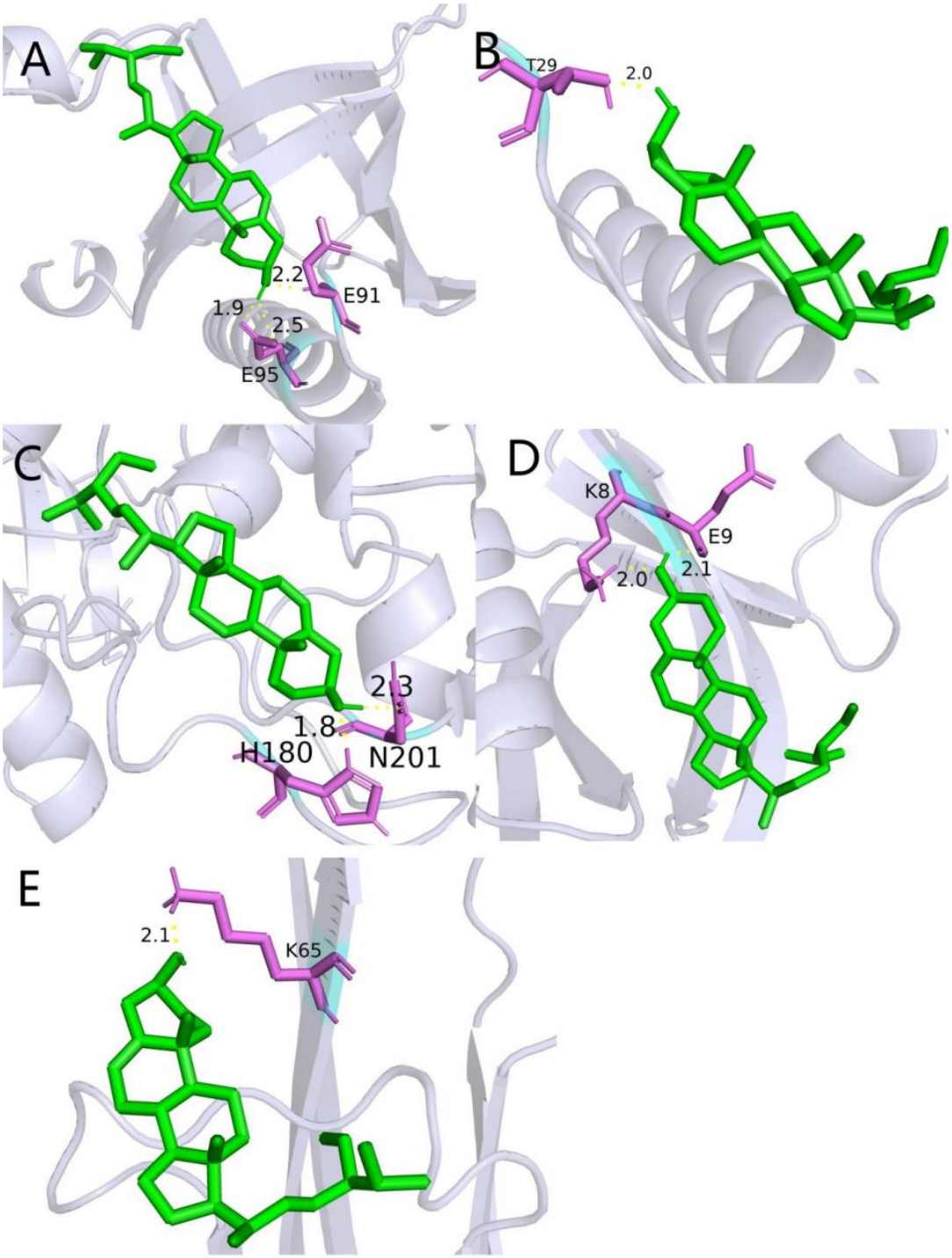

PIK3CG
CD40LG
NR3C1
ACHE
DPP4
CRP
XDH
HSPA5
NOS2
MMP3
CHEK2
GSTP1
SLC6A3
ESR2
NFE2L2
CACNA1S
MMP1
NR3C2
GSK3B
CYP3A4
MAPK14
NCF1
CASP8
ADH1C
PPARG
CHUK
STAT1
PTEN
MMP2
CCNA2
CDKN1A
IL4
CASP3
CCND1
CDK2
AR
MAOB
EGF
CCL2
IL10
FN1
MAPK1
IL6
CYP1A2
SLC6A4
MPO
ERBB2
MMP9
IFNG
IL1B
AKT1
VCAM1
FASLG
TOP2A
CXCL10
CXCL8
ESR1
TNF
IL1A
HIF1A
COL1A1
CTSD
CASP9
TP53
BCL2
RELA
TGFB1
DRD1
RXRA
CAV1
ICAM1
IL2
FOS
HMOX1
PGR
CYP2C9
RAF1
HTR2A
HSF1
GSTM1
PRKCA
CYP2B6
CDK1
PPARA
IGF2
CYP1A1
BCL2L1
PRKCB
RB1
GJA1
KDR
RASA1
SELE
IGFBP3
HSPB1
SPP1
ERBB3
CYP1B1
BAX
ALOX5
INSR
NOS3
COL18A1
1
29


HLJDD
AD
hsa05140
hsa05165
hsa05022
hsa05163
hsa05162
hsa05200
hsa05164
hsa05142
hsa05170
hsa05417
hsa04210
hsa05160
hsa05169
hsa04657
hsa05145
hsa04668
hsa05161
hsa04932
hsa05167
hsa05152
AKT1 CASP9 CHUK CYP1B1 GABRA1 CACNA1S HSPB1 IL2 MMP3 PLAT PTEN
STAT1 NR3C1 XDH DRD5 ADH1C FASLG NOS2 ADRA2C ALOX5 CAV1 COL1A1
CYP3A4 GJA1 ICAM1 IL6 MMP9 PLAU RAF1 DRD3 TGFB1 HTR2A GSK3B
MAP2 CYP2B6 AR CCL2 CRP DPP4 GSTM1 IFNG INSR MPO PON1
RASA1 THBD GRIA2 CHRM1 HTR3A ADRA2A KDR PGR BAX CCND1 CTSD
EGF GSTP1 IGF2 KCNH2 NCF1 PPARA RB1 TNF CHRM2 ADRB1 IL4
ADRA2B OPRM1 MAPK14 BCL2 CD40LG CXCL10 ERBB2 HIF1A IGFBP3 MAOB NFE2L2
PPARG RELA TOP2A CHRM3 CDK2 SLPI APOD ABCA2 BCL2L1 CDK1 CXCL8
CA2 ERBB3 GABRA5 HMOX1 IL10 MAPK1 NOS3 PRKCA RXRA TP53 CHRM4
SLC6A2 ESR1 ADRA1A ACHE CASP3 CDKN1A CYP1A1 F7 HSF1 IL1A MMP1
NPEPPS PRKCB SELE VCAM1 CHRM5 SLC6A3 ESR2 ADRA1B NR3C2 ADRB2 CASP8
CHEK2 CYP1A2 FOS HSPA5 IL1B MMP2 PIK3CG PRSS1 SPP1 VEGFA DRD1
SLC6A4 CCNA2 CYP2C9 ADRA1D FN1
A1 HQ8 HQ7 ZZ6 ZZ1 HB9 HQ17 HQ16 HQ12 A6 A4 A3 HB13 HQ20 HQ5 HQ11 HQ3 HB4 HB2 HB12 A8 A2 B1 HQ2 HQ13 HL2 ZZ2 HB8 HB10 B2 HQ1 ZZ4 A5 HB11 HQ24 HQ18 HQ10 HB7 ZZ5 HB3 HQ15 HQ6 HQ4 HL3 HB6 HB1 HQ23 HQ9 HB15 HL1 A7 HB5 HQ21 HQ19 HQ14 ZZ3 HB14 HQ22


IFNG
IL1B
FN1
TP53
IL6
ESR1
MAPK1
TNF
CCND1
AKT1
12
29


GO- Enrichment
cellular response to nitrogen compound
response to hormone
cellular response to organic cyclic compound
celllular response to organonitrogen compound
response to inorganic substance
response to xenobiotic stimulus
response to peptide
cellular response to hormone stimulus
response to oxidative stress
response to reactive oxygen species
response to metal ion
celllular response to peptide
response to peptide hormone
cellular response to chemical stress
cellular response to oxidative stress
cellular response to peptide hormone stimulus
cellular response to xenobiotic stimulus
celllular response to reactive oxygen species
response to cadmium ion
cellular response to cadmium ion
BP
postsynapse
membrane raft
membrane microdomain
synaptic membrane
plasma membrane protein complex
dendritic tree
dendrite
postsynaptic membrane
receptor complex
presynapse
cell body
neuronal cell body
vesicle lumen
cytoplasmic vesicle lumen
secretory granule lumen
presynaptic membrane
glutamatergic synapse
plasma membrane raft
caveola
plasma membrane signaling receptor complex
CC
neurotransmitter receptor activity
G protein- coupled amine receptor activity
postsynaptic neurotransmitter receptor activity
adrenergic receptor activity
serotonin receptor activity
catecholamine binding
G protein- coupled serotonin receptor activity
G protein- coupled neurotransmitter receptor activity
alpha- adrenergic receptor activity
G protein- coupled acetylcholine receptor activity
acetylcholine receptor activity
phosphoric diester hydrolase activity
phospholipase activity
lipase activity
phospholipase C activity
phosphatidylinositol phospholipase C activity
epinephrine binding
alpha2- adrenergic receptor activity
alpha1- adrenergic receptor activity
adrenergic receptor binding
MF
Pathway
0.00
0.05
0.10
0.15
0.20
GeneRatio
- log10(pvalue)
40
30
20
10
count
10
20
30
40


KEGG- Enrichment
Pathway
Pathways in cancer
Lipid and atherosclerosis
Pathways of neurodegeneration - multiple diseases
Hepatitis B
Human papillomavirus infection
Human cytomegalovirus infection
Kaposi sarcoma- associated herpesvirus infection
Influenza A
Hepatitis C
Tuberculosis
Measles
Epstein- Barr virus infection
Toxoplasmosis
Non- alcoholic fatty liver disease
IL- 17 signaling pathway
TNF signaling pathway
Human immunodeficiency virus 1 infection
Chagas disease
Apoptosis
Leishmaniasis
0.10
0.15
0.20
GeneRatio
- log10(pvalue)
40
30
20
count
20
30
40


A
2.2
E91
1.9
2.5
E95
B
T29
2.0
C
2.3
1.8
H180
N201
D
K8
E9
2.0
2.1
E
2.1
K65