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A network pharmacology approach to identify the mechanisms and molecular targets of curcumin against Alzheimer disease

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Abstract

Background: Alzheimer disease (AD) is a degenerative brain disease, which may lead to severe memory loss and other cognitive disorders. However, few effective drugs are available in the clinic at present. Curcumin, a major ingredient of traditional Chinese medicine, Curcuma Longa, has various pharmacological activities. Therefore, exploring clinical drugs based on the inhibition of AD pathological features is imperative.

Methods: First, we utilized the HERB database and Swisstarget Prediction database to get the related targets of curcumin and intersected with the AD targets. The intersection targets were used to construct the protein-protein interaction network and performed gene ontology and kyoto encyclopedia of genes and genomes analyses. Further, we obtained targets of curcumin against AD-related tau and aβ pathology via the AlzData database. These targets were applied to perform GEO and receiver operating characteristic analyses. Finally, the reliability of the core targets was evaluated using molecular docking technology.

Results: We identified 49 targets of curcumin against AD, and kyoto encyclopedia of genes and genomes pathway enrichment analysis demonstrated that the Alzheimer disease pathway (has05010) was significantly enriched. Even more, we obtained 16 targets of curcumin-related Aβ and tau pathology. Among these targets, 8 targets involved the Alzheimer disease pathway and the biological process analyses showed that positive regulation of cytokine production (GO:0001819) was significantly enriched. Bioinformatic analyses indicated that HMOX1, CSF1R, NFKB1, GSK3B, BACE1, AR, or PTGS1 expression was significantly different compared to the control group in the AD patients. Finally, molecular docking studies suggested these genes have a good binding force with curcumin.

Conclusions: In this study, we identified curcumin exerted the effect of treating AD by regulating multitargets and multichannels through the method of network pharmacology.

Abbreviations: AD = Alzheimer disease, APP = amyloid precursor protein, AUC = area under the ROC curve, BP = biological processes, CC = closeness centrality, DL = drug-likeness, EGF = pro-epidermal growth factor, EU = Eucommia ulmoides cortex, FDA = Food and Drug Administration, GEO = Gene Expression Omnibus, GO = Gene ontology, Hacc = hydrogen bond acceptors, Hdon = hydrogen bond donors, KEGG = kyoto encyclopedia of genes and genomes, LD_{50} = lethal dose 50, MW = molecule weight, PPI = protein-protein interaction, Rbon = rotatable bonds, ROC = Receiver operating characteristic, TCM = traditional Chinese medicine, TCMSP = Traditional Chinese Medicine System Pharmacology Database, TPSA = topological polar.

Keywords: Alzheimer disease, curcumin, molecular docking, network pharmacology

1. Introduction

Alzheimer disease (AD) is the most prevalent form of dementia in the elderly, affecting more than 47 million people worldwide, and Alzheimer Disease International estimates that the number of people affected will reach 81.1 million in 2040.[1,2] China has one of the largest aged people globally, and the population aged 60 is estimated to increase from 15% to 36.5% in 2050.[3,4] Meanwhile, it has been reported that there are over 9 million AD patients in China now.[5] However, no preventive or curative treatment exists

XW and XZ have contributed equally to this work.

The authors declare that there are no competing interests associated with the manuscript.

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

The current analysis does not require ethical approval because our study only collects uploaded data information from the public database search. The article does not involve any patient personal data and does not cause harm to any patient.

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for AD at present, laying an enormous burden on public health and society.[6] AD is characterized by amyloid precursor protein (APP) derivative Aβ and microtubule-associated protein tau aggregation.[7] Moreover, AD is frequently accompanied by neuron loss.[8] Currently, there are limited treatment options available for AD, also, the new methods are still in their development stage, for example, optogenetics.[9] Until now, only 5 drugs have been approved by the Food and Drug Administration (FDA) to treat AD on market. But these drugs against AD can only ameliorate the AD's symptoms for a limited time do not prevent, delay, or halt the progression of the disease.[10–12] In addition, therapeutic agents for the treatment of AD are frequently associated with toxic side effects or loss of efficacy.[13,14] Therefore, exploring clinical drugs based on the inhibition of AD pathological features is imperative.

Recently, herbal medicines' natural products and active components for AD treatment have attracted great attention.[15] Curcumin is a principal curcuminoid of turmeric, long believed to be bioactive components, isolated from natural Curcuma longa Linn.[16–18] Curcumin and its metabolites possess anti-inflammatory,[19,20] antioxidant,[21,22] antimicrobial,[23] and antiviral[24,25] activities. Further, in recent years, the literature reports that curcumin has beneficial effects in diseases of the neurological system, including AD.[26,27] Curcumin suppresses the formation and promotes the disaggregation of amyloid-β plaques, attenuates the hyperphosphorylation of tau, and enhances its clearance. In summary, curcumin has the potential to be more effective than current treatments.[28]

Network pharmacology is a new field based on systems biology and combines polypharmacology, molecular network data, bioinformatics, and computer simulation.[29,30] The core idea of network pharmacology is therefore well adapted to analyze the multitargeted drugs, so network pharmacology methods may be appropriate for identifying the complex mechanisms of curcumin.[31] In this study thus, network pharmacology was used to elucidate the detailed anti-AD mechanisms of curcumin. Our approach is outlined in Figure 1. Further on, by using the AD database, we can filter out 16 targets involved in Aβ and tau pathology. With the help of high throughput technology and molecular docking, we verified the curcumin-related anti-AD targets.

2. Methods

2.1. Pharmacokinetic properties and toxicity prediction

The chemical structural formula of curcumin was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Then, the drug

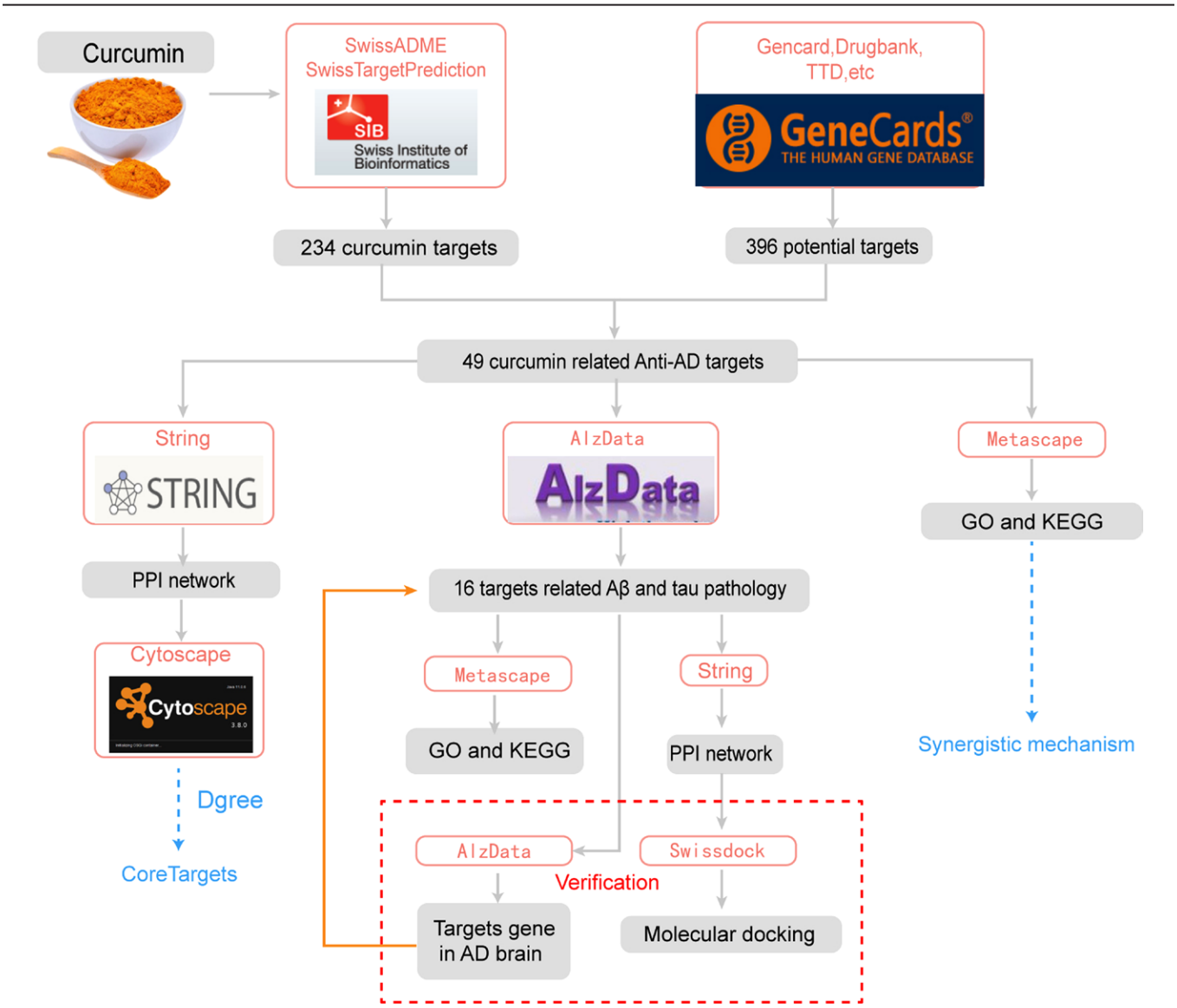

Figure 1. Study flowchart: overall study diagram.

likeness and the physicochemical properties were studied using SwissADME (http://www.swissadme.ch/), which was used to evaluate compounds' ADME. Additionally, We use ProTox-II (https://tox-new.charite.de/) to evaluate curcumin toxicity.

2.2. Prediction of curcumin targets

SwissTargetPrediction (www.swisstargetprediction.ch) is a web-based tool for target prediction of small bioactive molecules, that were employed to collect the curcumin-related targets. We imported the SMILES into SwissTargetPrediction to predict all potential targets of curcumin. Meanwhile, We utilized the HERB database (http://herb.ac.cn/) to get the related targets of curcumin for a comprehensive collection of drug targets, and the targets were derived from TCMID (http://www.megabionet.org/tcmid/) and TCMSP (https://tcmspw.com/tcmsp.php) databases. Next, the gene symbols of the potential target were obtained by searching the Uniprot databases (http://www.uniprot.org/uniprot/).

2.3. Predict targets of curcumin against AD

Using AD as a keyword, the AD gene was searched and screened by the following electronic databases: GeneCard database (https://www.genecards.org/), OMIM database (https://www.omim.org/), TTD database (http://db.idrblab.net/ttd/), PharmGKB database (https://www.pharmgkb.org/), DrugBank data (https://go.drugbank.com/). Then, through the UniProt database corrected their protein names as official names and defined species Source is human.

2.4. Screening of intersection targets

In order to clarify the interaction between AD-related targets and potential targets of curcumin, we intersected the AD targets and curcumin targets and drew the Venn diagrams with an online Venn diagram tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). After this, the Panther Classification System performed protein classification of the curcumin-related anti-AD targets (http://www.pantherdb.org/).

2.5. Construction and analysis of PPI network

The Protein-protein interaction (PPI) network interaction was obtained by introducing these potential therapeutic targets of AD to the STRING database (https://string-db.org) with the species limited as "Homo sapiens." Within each network, all interactions had confidence scores ≥ 0.4 (medium + high confidence). Thereafter, this network was imported to Cytoscape (Version 3.8.0) for visualization. The Cytoscape software is available for download through the Cytoscape website (https://cytoscape.org/). The degree was calculated to identify core targets by the NetworkAnalyzer plugin (http://apps.cytoscape.org/apps/networkanalyzer). A higher degree value node represented putative crucial targets of curcumin in the PPI network. The Transparency and size of a node represent the combined score. The top 10 targets were selected according to the degree as core targets.

2.6. GO and kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis

The visualization of GO and KEGG pathway enrichment analysis for green module genes used R package clusterProfiler 28 from the Bioconductor project. The level of significance was set to $P < .05$ for all the tests. The top relevant enriched GO and KEGG terms were visualized using an online tool (http://www.bioinformatics.com.cn/). Another online tool we used was the RAWGraphs (https://app.rawgraphs.io/) for drawing Sankey diagram about the targets–pathways relationship.

2.7. Analysis of curcumin targets related to AD pathology

Firstly, gene symbols for the target proteins of curcumin against AD were given as input to the AlzData (http://www.alzdata.org/) database for correlation analysis of AD pathology (Aβ and tau). Then collect the results obtained from Microsoft Excel. And these targets of the curcumin related to AD pathology were used for subsequent data analysis, such as GO and KEGG pathway enrichment analyses. To compare the expression level of the risk genes in AD cases with healthy controls, we performed the differential expression analysis using the comprehensive AlzData database. Data were visualized using GraphPad Prism (version 9.0) and Flourish (https://app.flourish.studio/). Values were shown as mean ± SD.

2.8. Molecular docking

In this study, molecular docking was performed using AutoDockTools-1.5.6, and the crystal structure was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/), the docking procedure was repeated 3 times. The curcumin molecular structure was retrieved from the PubChem database. Docking results were analyzed using LigPlot+ (http://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/) and PyMol (Version 4.6.0), and the interactions were further visualized using LigPlot.

3. Results

3.1. The pharmacokinetic properties and toxicity prediction of curcumin

The structural information of curcumin was obtained from PubChem (Fig. 2), and the relevant ADME information was obtained from swissADME and TCMSP. Table 1 shows the SwissADME predicted pharmacokinetic of the curcumin, including topological polar surface area and Lipinski rule

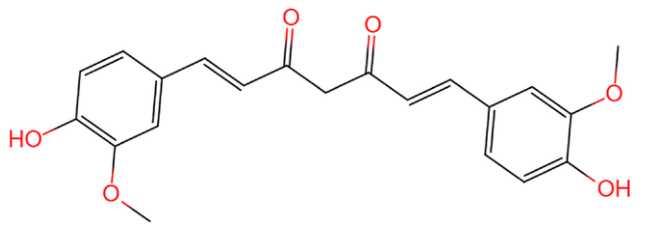

Figure 2. Structure of curcumin.

Table 1

Pharmacological and molecular properties of curcumin.

Name	Curcumin
MW	368.4
Formula	$C_{21}H_{20}O_6$
Hdon	2
Hacc	6
Rbon	8
TPSA (Å)	93.06
DL	Good
Lipinski	Yes
GI absorption	High
Caco-2 Permeability	−4.834
BBB permeant	No
T1/2	0.948
Log Kp (skin permeation)	−6.28 cm/s
Clearance	13.839 mL/min/kg

BBB = blood-brain barrier, DL = drug-likeness, GI = gastrointestinal absorption., Hacc = hydrogen bond acceptors, Hdon = hydrogen bond donors, MW = molecule weight, Rbon = rotatable bonds, TPSA = topological polar.

of 5. The results showed that curcumin complies with Lipinski rule of 5 and is predicted to have a good drug-likeness. The ProTox-II software was used for the evaluation of toxicological parameters in silico (Table 2).[32] The results demonstrated that curcumin showed no observable toxicity, except for Immunotoxicity.

3.2. Screening of targets of curcumin against AD

To identify the curcumin-associated targets, 233 targets were collected from SwissTargets and Herb. In addition, A total of 396 disease-related targets were obtained from GeneCard, DrugBank, TTD, OMIM, and PharmGBK. Based on the above results, we identified 49 targets of curcumin against AD by the intersection of 233 curcumin-associated targets and 396 disease-related targets (Fig. 3A). Detailed information about these intersection targets is shown in Table 3. Moreover, these 49 genes were classified into 11 distinct classes using the Panther Classification System (Fig. 3B). The top 5 protein classes were protein modifying enzyme (22.50%), metabolite interconversion enzyme (20.00%), intercellular signal molecule (12.50%), transmembrane signal receptor (12.50%), gene-specific transcriptional regulator (10.00%).Among the protein modifying enzymes and metabolite interconversion enzymes, MTOR, GSK3B, AKT1, and MAPK1 are nonreceptor serine/threonine-protein kinase; ADAM17, BACE1, and CASP3 are protease; PTGS1, PTGS2, HSD11B1, AR, MAOA, HMOX1 are oxidoreductase.

3.3. Construction and analysis of PPI network of curcumin

The PPI network was constructed using the Cytoscape based on the obtained PPI relationships (Fig. 3C). The degree was calculated by the NetworkAnalyzer, and the top 10 targets were selected as core targets. The top 10 targets were; AKT1, IL6, TNF, IL1B, EGFR, CASP3, TP53, VEGFA, PTGS2, APP (Fig. 3D). Taken together, the above results imply these core targets may Play a Critical Role in AD treatment.

3.4. The GO biological process enrichment analysis

GO enrichment analysis mainly involves 3 aspects: cell composition, molecular function (MF), and biological process (BP).[33]

Table 2

Toxicity of curcumin.

LD_{50}	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
2000 mg/kg	Inactive	Inactive	Active	Inactive	Inactive

LD_{50}, lethal dose 50.

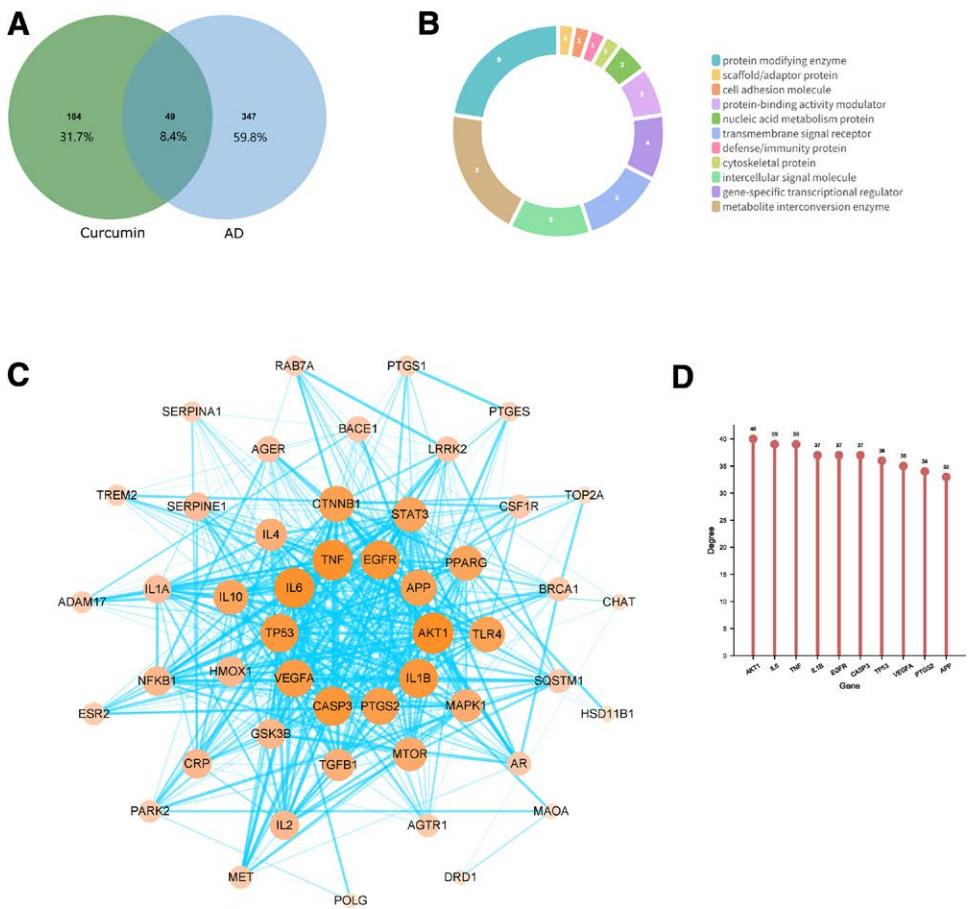

Figure 3. (A) The Venn diagram is the intersection of key AD targets and curcumin targets. (B) The protein classification of targets of curcumin against AD using the PANTHER classification system. (C) PPI networks of curcumin against AD-related targets. (D) Core targets degree score of targets of curcumin against AD. AD = Alzheimer disease, PPI = protein-protein interaction.

Table 3

Targets of curcumin against AD.

Number	Gene ID	Protein description	Gene symbol	Number	Gene ID	Protein description	Gene symbol
1	4128	Monoamine oxidase A	MAOA	26	7124	Tumor necrosis factor	TNF
2	351	Amyloid-beta precursor protein	APP	27	1103	Choline O-acetyltransferase	CHAT
3	9536	Prostaglandin E synthase	PTGES	28	8878	Sequestosome 1	SQSTM1
4	23,621	Beta-secretase 1	BACE1	29	1499	Catenin beta 1	CTNNB1
5	7153	DNA topoisomerase II alpha	TOP2A	30	5428	DNA polymerase gamma, catalytic subunit	POLG
6	5742	Prostaglandin-endoperoxide synthase 1	PTGS1	31	836	Caspase 3	CASP3
7	1956	Epidermal growth factor receptor	EGFR	32	4233	MET proto-oncogene, receptor tyrosine kinase	MET
8	6774	Signal transducer and activator of transcription 3	STAT3	33	7422	Vascular endothelial growth factor A	VEGFA
9	3290	Hydroxysteroid 11-beta dehydrogenase 1	HSD11B1	34	5468	Peroxisome proliferator activated receptor gamma	PPARG
10	207	AKT serine/threonine kinase 1	AKT1	35	7040	Transforming growth factor beta 1	TGFB1
11	2932	Glycogen synthase kinase 3 beta	GSK3B	36	3558	Interleukin 2	IL2
12	5054	Serpin family E member 1	SERPINE1	37	3586	Interleukin 10	IL10
13	185	Angiotensin II receptor type 1	AGTR1	38	672	BRCA1 DNA repair associated	BRCA1
14	6868	ADAM metallopeptidase domain 17	ADAM17	39	3565	Interleukin 4	IL4
15	1436	Colony stimulating factor 1 receptor	CSF1R	40	1401	C-reactive protein	CRP
16	2475	Mechanistic target of rapamycin kinase	MTOR	41	5594	Mitogen-activated protein kinase 1	MAPK1
17	5071	Parkin RBR E3 ubiquitin protein ligase	PRKN	42	7157	Tumor protein p53	TP53
18	3162	Heme oxygenase 1	HMOX1	43	2100	Estrogen receptor 2	ESR2
19	4790	Nuclear factor kappa B subunit 1	NFKB1	44	120892	Leucine rich repeat kinase 2	LRRK2
20	7099	Toll-like receptor 4	TLR4	45	7879	RAB7A, member RAS oncogene family	RAB7A
21	54,209	Triggering receptor expressed on myeloid cells 2	TREM2	46	5265	Serpin family A member 1	SERPINA1
22	5743	Prostaglandin-endoperoxide synthase 2	PTGS2	47	1812	Dopamine receptor D1	DRD1
23	3552	Interleukin 1 alpha	IL1A	48	177	Advanced glycosylation end-product specific receptor	AGER
24	3553	Interleukin 1 beta	IL1B	49	367	Androgen receptor	AR
25	3569	Interleukin 6	IL6			Tumor necrosis factor	

GO enrichment entries of the BP show the top 20. Within the BP categories, positive regulation of cytokine production (GO:0001819), regulation of inflammatory response (GO:0050727), positive regulation of DNA-binding transcription factor activity (GO:0051091), response to molecule of bacterial origin (GO:0002237), and response to lipopolysaccharide (GO:0032496) were dominant terms (Fig. 4A). This result illustrates that curcumin against AD involved a variety of targets and was involved in several BPs.

3.5. KEGG pathway analysis to explore the potential mechanisms of curcumin in treating AD

After KEGG analysis, 80 KEGG pathways were predicted, and the top 20 enriched KEGG pathways are shown (Fig. 4B). The majority of the selected subpathways we discuss belong to 5 KEGG pathways: AD (hsa05010), PI3K-Akt signaling pathway (hsa04151), Human cytomegalovirus infection (hsa05163), AGE-RAGE signaling pathway in diabetic complications (hsa04933), and Proteoglycans in cancer (hsa05205). The details of all KEGG pathway enrichment analyses were shown in (Table 1, Supplemental Digital Content 1, http://links.lww.com/MD/H78). The targets in the AD pathway are shown in the mechanistic diagram of AD pathology (Fig. 4C). And Fig. 5 presents these targets involved in the top 5 KEGG pathways.

3.6. Bioinformatics analysis of targets of curcumin against AD

In order to analyze the potential targets of curcumin-related Aβ and tau pathology, using the Alzdata database. Of these 49, 16 targets were significantly correlated with tau, Aβ, or both Aβ and tau (Fig. 6A). Out of these 16 targets, 15 targets were used to construct the PPI network (Fig. 6B). Next, these targets were ranked according to the degree score, IL1B, VEGFA, STAT3, GSK3B, IL1A, TGFB1, HMOX1, NFKB1, ADAM17, and AGER were predicted as core targets (Fig. 6C). Under the biological process in AD pathology related targets, positive regulation of cytokine production (GO:0001819), response to molecule of bacterial origin (GO:0002237), regulation of DNA-binding transcription factor activity (GO:0051090), mononuclear cell differentiation (GO:1903131), temperature homeostasis (GO:0001659), and others were significantly enriched (Fig. 6E). KEGG pathway enrichment analysis of these targets showed that the enrichment genes of the AD (hsa05010) pathway were the most enriched (Fig. 6F). Meanwhile, We summarized these enrichment genes in Figure 6D.

3.7. Targets of curcumin against AD-related tau and aβ pathology based on analysis of gene expression omnibus (GEO)

Considering that the expression level of target genes might change and contribute to AD risk, we further investigated whether targets related to AD pathology are differentially expressed in AD patients compared to healthy controls by using the "Differential expression" module of the AlzData database. Among the temporal cortex, HMOX1, CSF1R, and NFKB1 were significantly upregulated (Fig. 7A–C), GSK3B and BACE1 were significantly downregulated (Fig. 7D,E) in AD patients compared to controls. Among the entorhinal cortex, AR, HMOX1, and NFKB1 were upregulated considerably (Fig. 7F–H), but not a single gene was significantly downregulated in AD patients compared to controls. Similarly, AR, PTGSE, and NFKB1 were significantly upregulated (Fig. 7I–K), but no gene exhibited significant downregulation in the hippocampus. These results indicate that targets of curcumin might play a significant role in AD pathogenesis.

3.8. Targets of curcumin against AD-related tau and aβ pathology based on analysis of receiver operating characteristic

Receiver operating characteristic (ROC) analysis was performed to assess the accuracy of curcumin targets related to AD Pathology in AD diagnosis. The area under the ROC

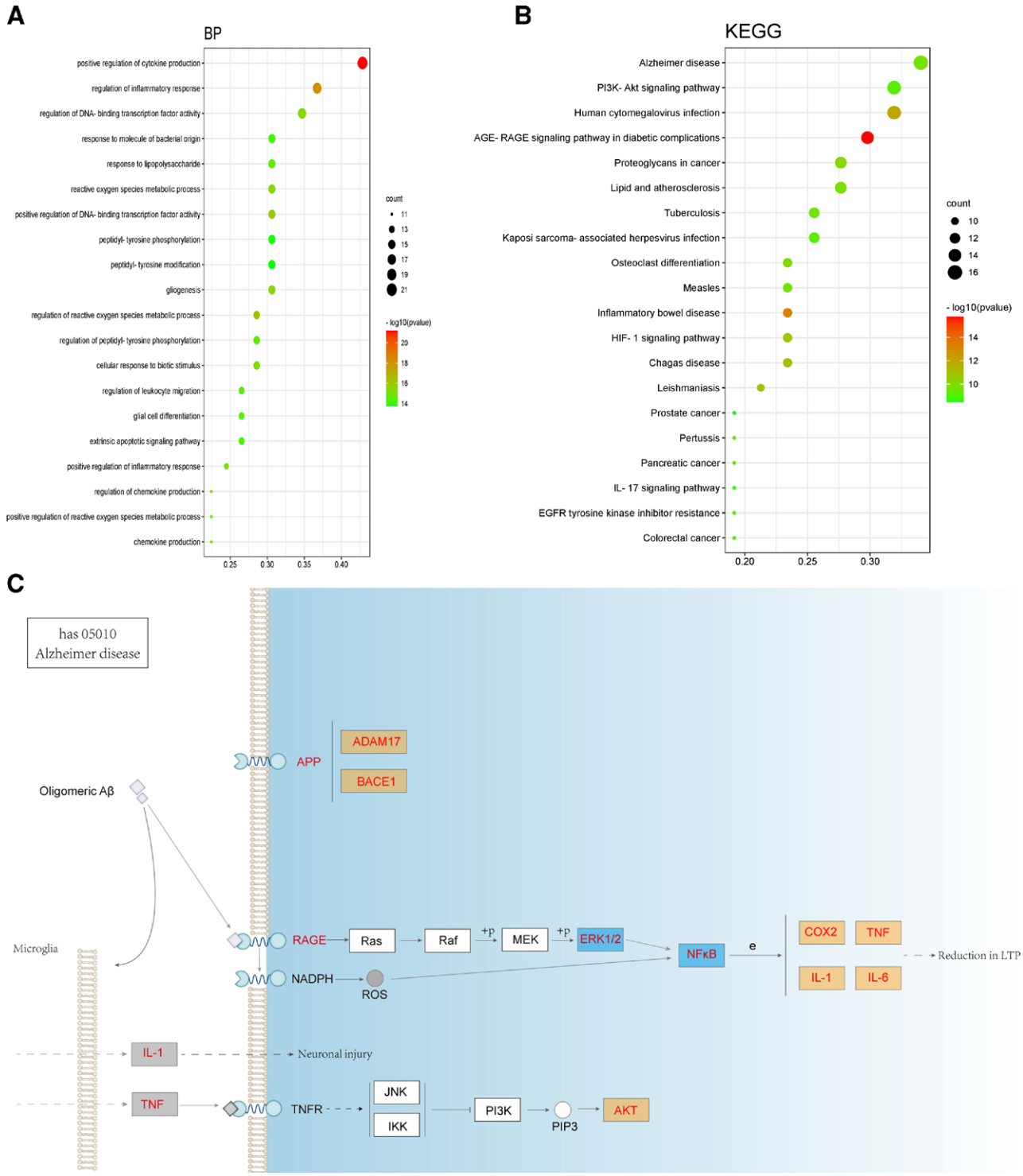

Figure 4. (A) The top 20 biological process analyses of GO. (B) The top 20 pathways enriched in KEGG. (C) Genes associated with Alzheimer disease are Presented in a mechanistic diagram of AD pathology. AD = Alzheimer disease, GO = gene ontology, KEGG = kyoto encyclopedia of genes and genomes.

curve (AUC) is used to assess the global performance of a prediction method. And closing the AUC score is to 1, the better the diagnostic performance is. NFKB1 (AUC = 0.733), HMOX1 (AUC = 0.643), GSK3B (AUC = 0.685), CSF1R (AUC = 0.630), and BACE1 (AUC = 0.672) achieve a satisfactory level of area under curve values (Fig. 8A–E). The highest AUC value was obtained for NFKB1. Hence, indicative of good prediction accuracy for these targets in AD diagnosis.

3.9. Molecular docking of targets of curcumin against AD-related tau and aβ pathology

To elucidate the binding mode of the curcumin to targets of curcumin against AD-related tau and aβ pathology, molecular docking studies were performed. The results of the final molecular docking study are shown in Table 4. In molecular docking, CSF1R revealed the lowest dock score of – 9.4 kcal/mol and exhibited as the most potential binder of targets of curcumin against AD-related tau

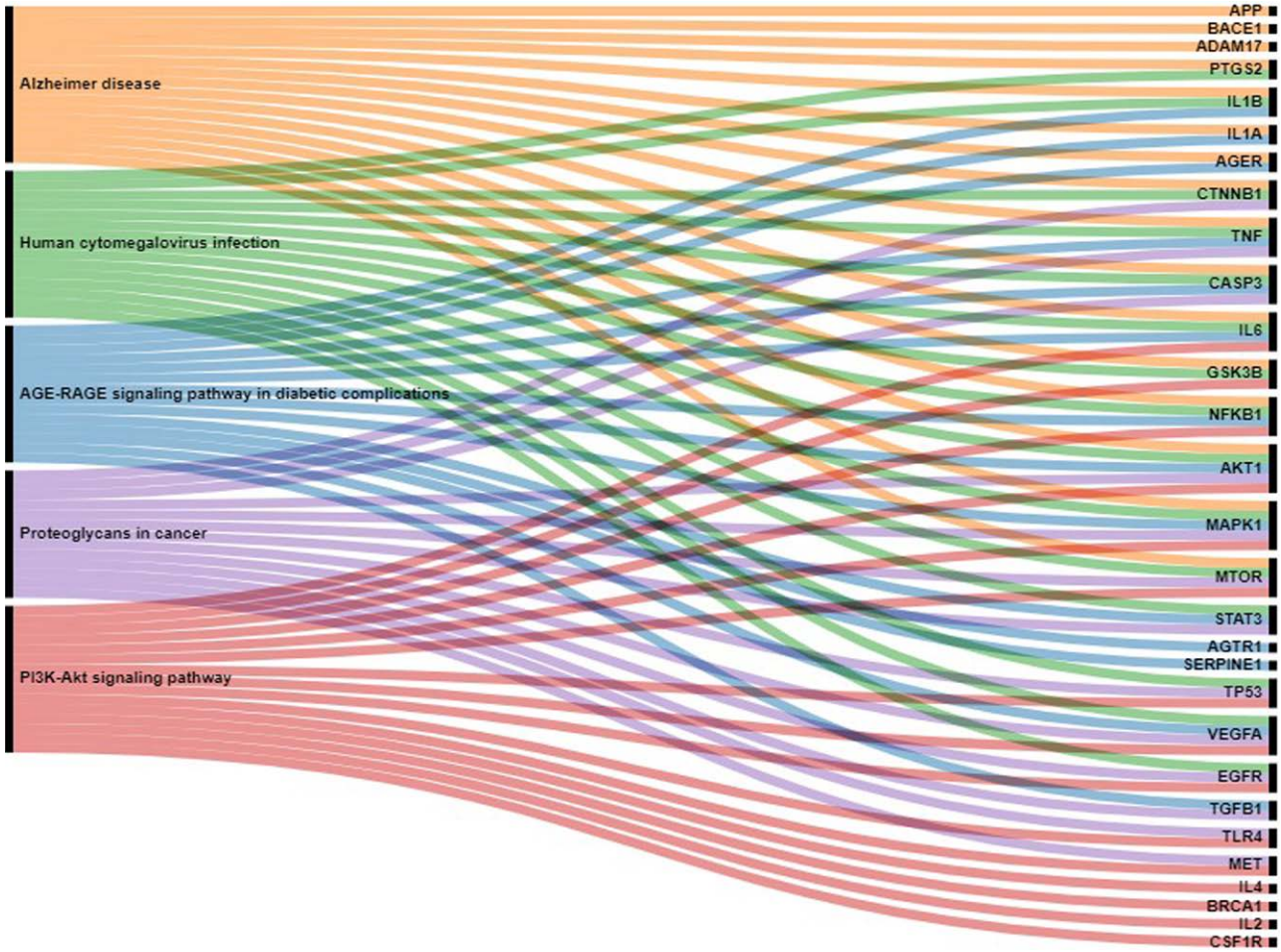

Figure 5. The genes involved in the top 5 pathway.

and aβ pathology. And the rest of target (AR, BACE1, HMOX1, AGER, GSK3B, IL1B, STAT3, NFKB1) dock score were –8.4 kcal/mol, –7.9 kcal/mol, –7.6 kcal/mol, –7.2 kcal/mol, –7.1 kcal/mol, –6.7 kcal/mol, –6.7 kcal/mol, –5.4 kcal/mol, respectively (Table 4). The molecular docking study showed that hydrogen bonds were the main linkage between curcumin and these target proteins. The LIGPLOT showed the existence of hydrogen bonds between curcumin and some residues of target protein (Fig. 9). Molecular docking revealed that curcumin was bound to CSF1R via Cys666 and Arg801, with hydrogen bond distances of 3.10 and 3.24 Å, respectively. The distance of 2 hydrogen bonds between curcumin and Thr663 were 2.87 and 3.12 Å, respectively (Fig. 9A). Curcumin bound with AR by Lys808, with hydrogen bond distances of 3.02 Å (Fig. 9B). Curcumin was bound to BACE1, forming 5 hydrogen bonds at Ser325, Thr72, and Gly34 (Fig. 9C). Curcumin bound with HMOX1 by Arg136, with hydrogen bond distances of 2.99 Å (Fig. 9D). For curcumin and AGER, a ring forms 2 hydrogen bonds with Asp73 and Arg77 (Fig. 9E). Curcumin bound with GSK3B by Arg344, with hydrogen bond distances of 2.95 Å (Fig. 9F). And curcumin bound with IL1B by Ser125, with hydrogen bond distances of 2.83 Å (Fig. 9G). Curcumin was bound to STAT3, forming 1 hydrogen bond at Gln247, with hydrogen bond distances of 3.05 Å (Fig. 9H). In addition, curcumin was bound with NFKB1 by Lys85, with hydrogen bond distances of 3.12 Å (Fig. 9I).

4. Discussion

To explore the mechanisms and molecular targets of curcumin against AD mechanisms, we performed network pharmacology and database mining in this study. Network pharmacology is based on the analysis of network models and systems biology, which is a comprehensive approach based on traditional pharmacology, bioinformatics, chemoinformatics, and network biology.[34,35] Curcumin is usually found in many natural products, such as Fructus tsaoko, Rhizoma Curcumae longae, Rhizoma Curcumae. TCM databases, such as TCMSP, TCMID, TCM-MeSH are applied for predicting the active ingredients of these natural products. Because of the issue of bioavailability with the curcumin (OB = 5.15%), It is often not screened for as an active ingredient. However, available studies indicate that curcumin has been widely used in neurodegenerative diseases.[36–38] A recent study indicated that curcumin could improve lipid metabolic disorders by the ATP binding cassette A1 transmembrane transport system in AD.[39] In addition, curcumin improves parkinsonian disability scores in vivo and inhibits PC12 cell death in vitro by inhibiting AKT/mTOR signaling pathway, which is mediated by autophagy.[40] Consequently, we selected curcumin for all our subsequent experiments.

In the present study, we first determined the pharmacokinetic properties and toxicity of curcumin via electronic databases. Curcumin compliance with Lipinski rule of 5, and with a higher LD50 value. The literature has previously reported intraperitoneal administration of curcumin has shown LD50 in the mouse at 1500 mg/kg,[41] which is close to the predicted result by the electronic databases. Afterward, a total of 49 targets of curcumin against AD targets were obtained. In the curcumin-AD PPI network, the top 10 targets were screened according to the degree, and they are AKT1, IL6, TNF, IL1B, EGFR, CASP3, TP53, VEGFA, PTGS2, and APP. Therein, The top-ranking

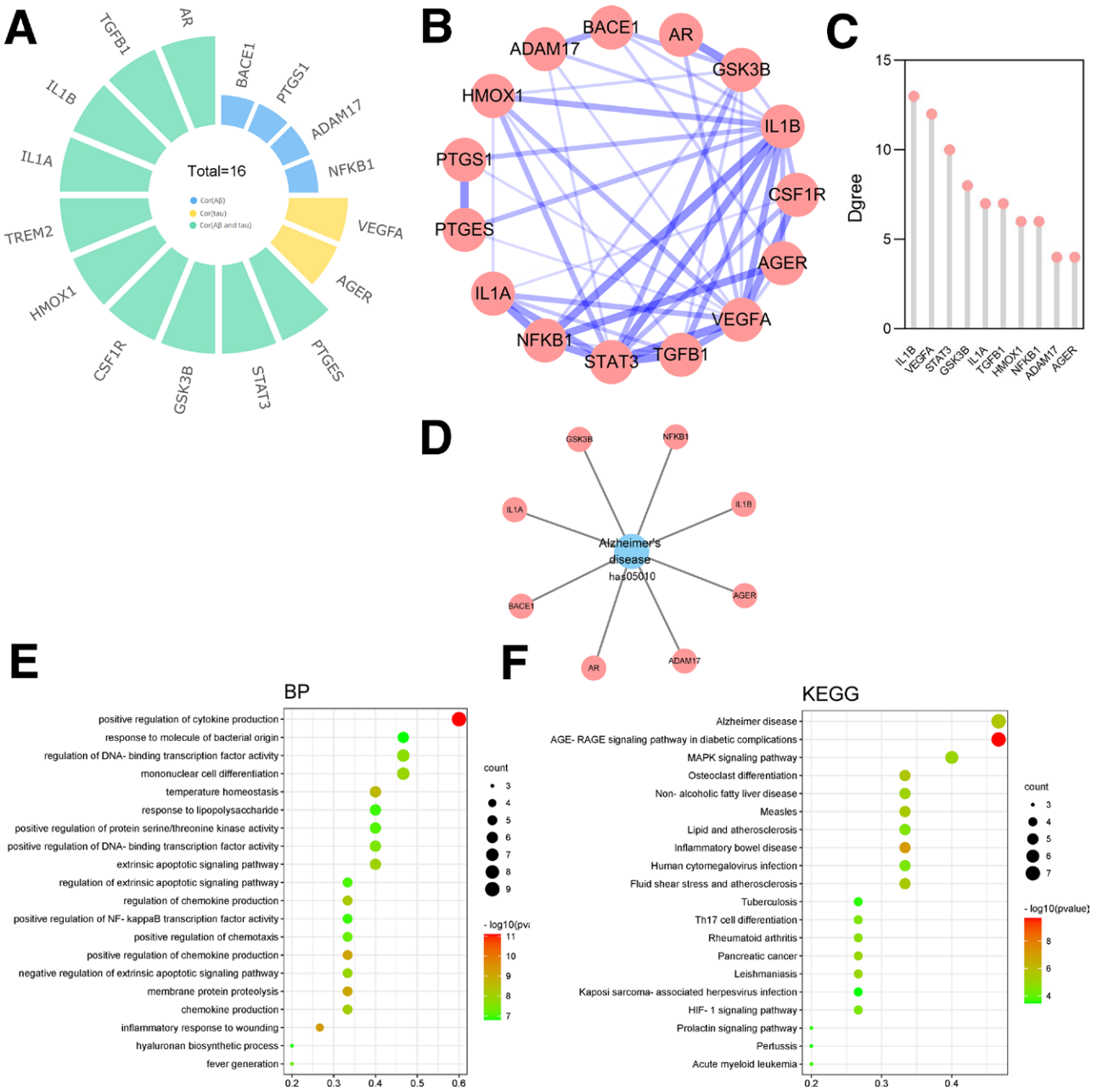

Figure 6. (A) Targets of curcumin significantly related Aβ, tau, or Aβ and tau. (B) PPI networks of targets of curcumin-related Aβ and tau pathology. (C) The top 10 core targets from the PPI network were ranked by degree. (D) Targets correlated Alzheimer disease pathway. (E) GO enrichment analysis of targets of curcumin-related Aβ and tau pathology. (F) KEGG pathway enrichment analysis of targets of curcumin-related Aβ and tau pathology. GO = gene ontology, KEGG = kyoto encyclopedia of genes and genomes, PPI = protein-protein interaction.

target was AKT1. AKT1 is a serine/threonine-protein kinase that plays a vital role in controlling cell survival and apoptosis.[42] Arvanitakis et al 2020 reported that brain AKT phosphorylation is the key node of insulin and other growth factors, which is related to AD neuropathology and cognitive dysfunction.[43] In addition, some reports indicate that ROS-mediated oxidative modification of AKT1 leads to synaptic dysfunction in AD, seen as the loss of activity-dependent protein translation critical for synaptic plasticity and maintenance.[44] Multiple AD-related network pharmacology literature reported that AKT1 plays a crucial role in AD.[43–47] IL6, TNF, and IL1B are often used as proinflammatory markers in AD.[48,49] The remaining core targets are all playing essential roles in AD.[50–54]

After KEGG enrichment, KEGG pathways mainly were concentrated on AD signaling pathway. AGER, IL1A, IL1B, NFKB1, PTGS2, NFKB1, TNF, and MAPK1 involve the RAGE-mediated NF-κB signaling pathway via the ligation of Aβ to RAGE. It has been reported that suppressors of neuroinflammation exert protective effects on Aβ-induced inflammatory response by blocking Aβ binding to RAGE and suppressing the RAGE-mediated signaling pathway.[55,56] TNF and AKT1 involve the TNF-activating cell signaling pathways, including JNK, MAPK, and PI3K/AKT. In the BP, the GO terms positive regulation of cytokine production (GO:0001819), regulation of inflammatory response (GO:0050727), positive regulation of DNA-binding transcription factor activity (GO:0051091) were significantly enriched. This may hint at curcumin may exert anti-AD effects through multiple pathways and multiple BPs.

After that, we obtained 16 targets of curcumin against AD-related tau and aβ pathology by the AlzData database.

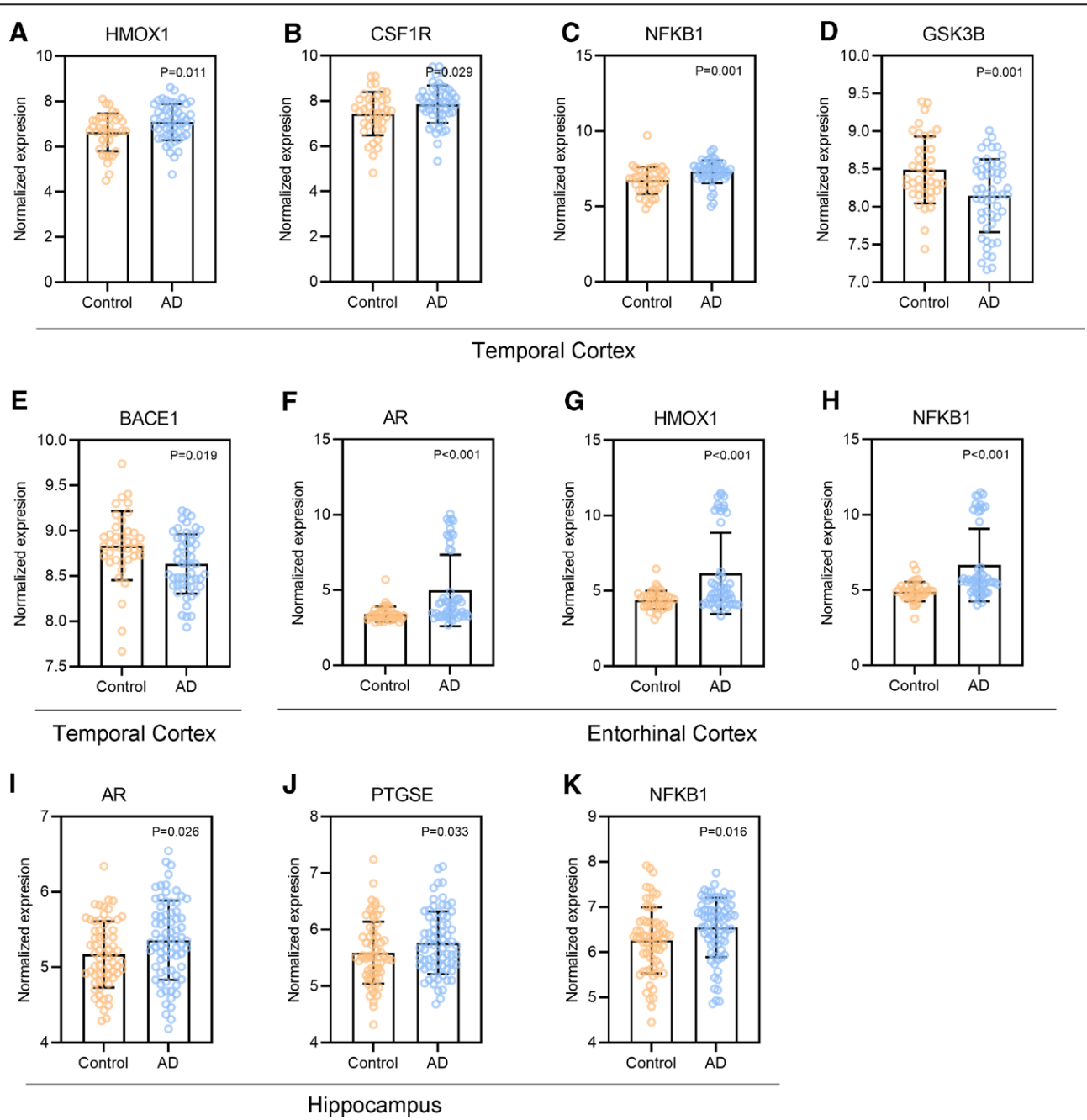

Figure 7. (A–E) Differential gene expression compared to the control group in the temporal cortex (F–H) Differential gene expression compared to the control group in the entorhinal cortex. (F–H) Differential gene expression compared to the control group in the hippocampus. Entorhinal cortex, n = 39 in each group. Hippocampus, n = 66 in the healthy control group, n = 74 in the AD group. Temporal cortex, n = 39 in the healthy control group, n = 52 in the AD group Data are presented as means ± standard errors of the mean (SEM). AD = Alzheimer disease

Among these targets, We focused on analyzing the targets related to the AD signaling pathway (e.g., GSK3B, NFKB1, IL1B, IL1A, ADAM17, AGER, AR, BACE1). ADAM17 is a transmembrane metalloproteinase, and ADAM17 activation may counteract the Aβ aggregation and cognitive deterioration.[57,58] Additionally, ADAM17 has been suggested to be regulatory for APP α-Secretase. Enhanced activity of ADAM17 leads to neuroprotective soluble APP α Increased secretion of the fragment and Aβ Of production, which may benefit this disease.[59] GSK3B is a known drug target for AD, which regulates several different cellular processes, and GSK3B dysregulation has been implicated in the pathogenesis of both AD.[60,61] Some investigators found that curcumin may inhibit Aβ-induced tau hyperphosphorylation involving PTEN/Akt/GSK-3β pathway, holding great potential in improving targeted drug delivery and the recovery of neuronal function in AD therapy.[62,63] NFKB1 is a transcriptional regulator that can be activated by various intracellular and extracellular stimuli, such as cytokines, oxidative radicals, UV irradiation, and bacterial or viral products.[64] The link between curcumin and NFKB1 has been studied extensively. In our research, NFKB1 expression was upregulated significantly compared to the control group in the temporal cortex, entorhinal cortex, and hippocampus of AD patients. In the previous study, administration of curcumin 100 mg/kg to lipopolysaccharide-induced memory impairment mice, the results demonstrated that curcumin inhibited the upsurge of NF-κβ transcript and protein levels, thus inhibiting TNF-α, IL-1β, COX-2, and iNOS. This ultimately decreased

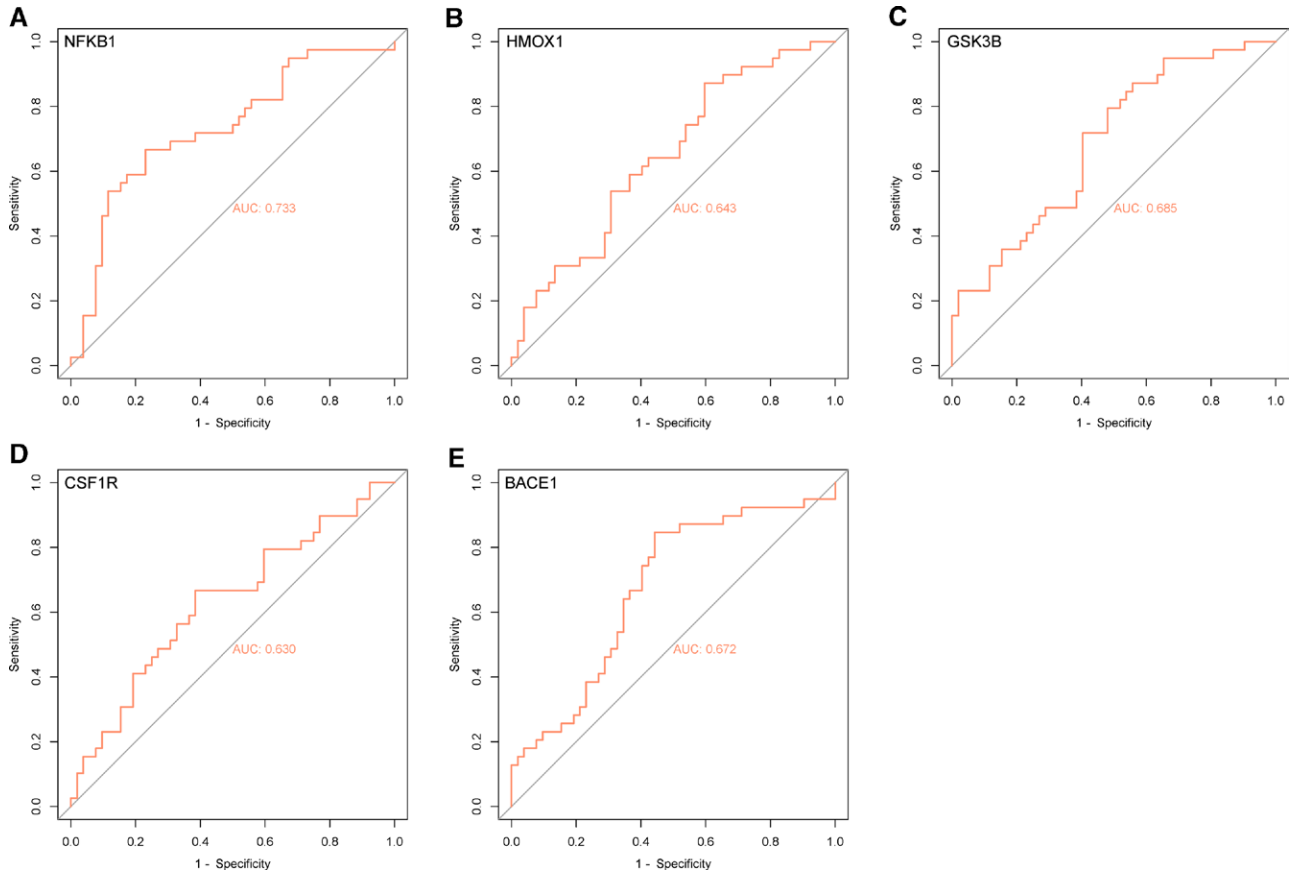

Figure 8. (A–E) The ROC curve of targets of curcumin against AD-related tau and aβ pathology. AD = Alzheimer disease, ROC = receiver operating characteristic.

Table 4

Molecular docking of curcumin with Aβ and tau pathology associated AD targets.

Ligands	Target	PDB	Delta G (kcal/mol)
Curcumin	CSF1R	2I1M	−9.4
Curcumin	AR	1E3G	−8.4
Curcumin	BACE1	1TQF	−7.9
Curcumin	HMOX1	6EHA	−7.6
Curcumin	AGER	2E5E	−7.2
Curcumin	GSK3B	1GNG	−7.1
Curcumin	IL1B	1HIB	−6.7
Curcumin	STAT3	6NJS	−6.7
Curcumin	NFKB1	1MDI	−5.4

Aβ deposition and eventually reduced inflammation and apoptosis.[65] Moreover, in vitro study showed that curcumin suppressed NF-κB and its downstream proinflammatory targets including COX-2, and iNOS in N2a/APPswe cells.[66] These results implicate NFKB1 might be an anti-AD target of curcumin. These results are consistent with the findings of our study. CSF1R is a key tyrosine kinase transmembrane receptor regulating microglia homeostasis, neurogenesis, and neuronal survival in the central nervous system.[67] However, few studies evaluated the association between CSF1R and curcumin. Currently, our analysis of GEO datasets showed that CSF1R expression was increased in the temporal cortex of the AD group. These results implicate CSF1R might be an anti-AD target of curcumin.

The results of molecular docking showed that curcumin exhibited preferable docking with CSF1R, and 3 hydrogen bonds were formed, followed by AR, BACE1, HMOX1, AGER, GSK3B, IL1B, STAT3, and NFKB1, respectively. Molecular docking showed that curcumin have high binding activity (the best free binding energy were all <−5.0 kcal/mol) to the predicted target protein and that the anti-AD effects of curcumin are closely related to these targets. This further illustrates that curcumin can stably bind to the protein receptor encoded by the core target gene and play its role in the treatment of AD. These results validated our network pharmacology analysis, and these factors can form the basis for the mechanistic understanding of AD pathogenesis in future studies and serve as viable targets for therapeutic development.

In summary, in this study, we analyzed the mechanisms of action of curcumin on AD and identified the core targets and pathways of curcumin against AD through a network pharmacology method. Our results suggest that GSK3B and NFKB1 could be regarded as valuable targets for AD treatment, curcumin can systematically improve the pathological features of stroke through multifactorial, multitarget, and multipathway. These results will provide better evidence for future clinical decision-making.

Contributions

Conceptualization: Xinyan Wu, Xiaomei Zheng.
Data curation: Xinyan Wu, Xiaomei Zheng.
Formal analysis: Xinyan Wu, Huaqiao Tang.
Funding acquisition: Gang Ye.
Investigation: Xinyan Wu.
Methodology: Ling Zhao, Xinyan Wu.
Project administration: Changliang He, Yuanfeng Zou, Xiaomei Zheng.
Resources: Xu Song, Lixia Li.
Software: Zhongqiong Yin, Gang Ye.

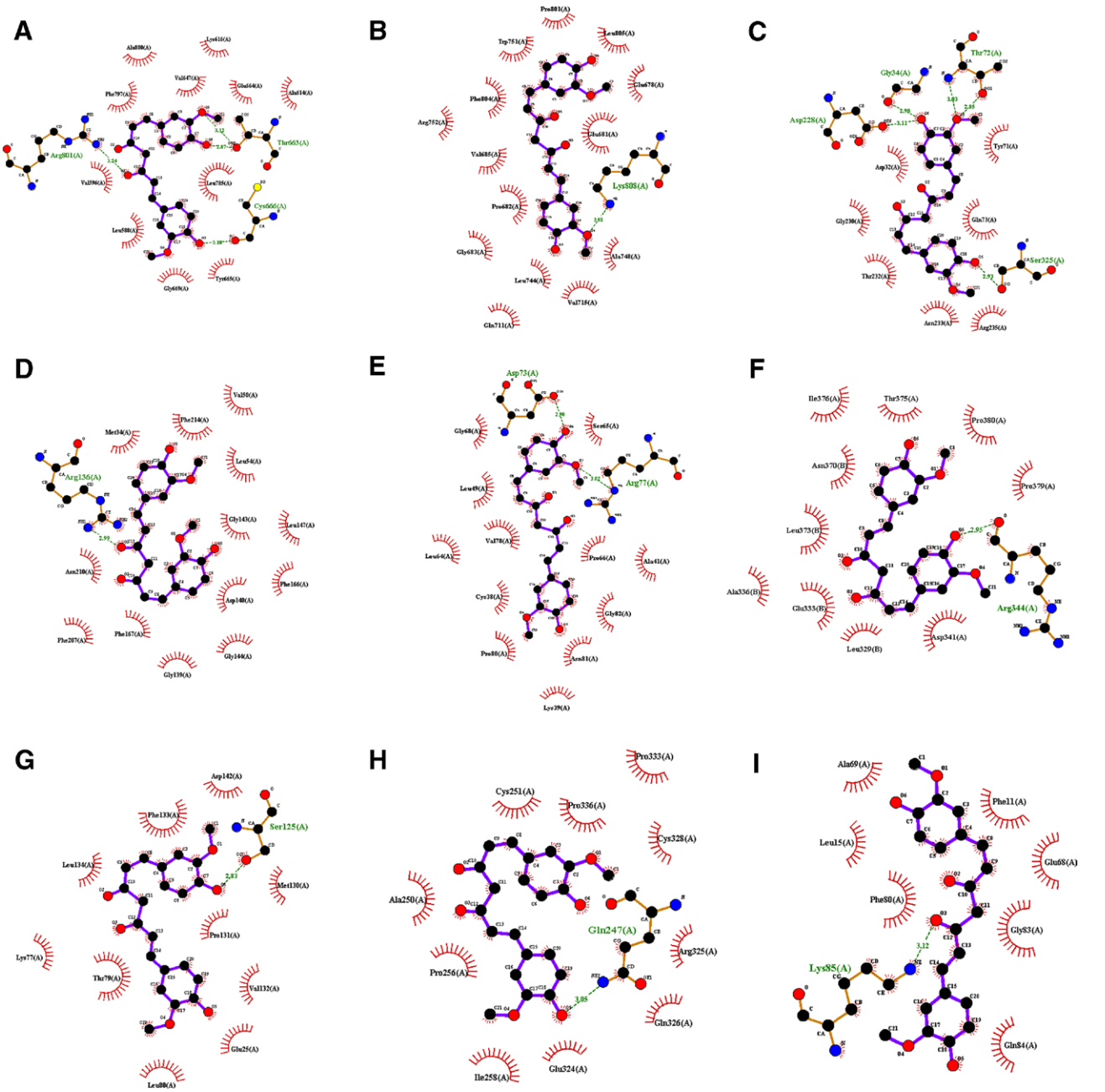

Figure 9. Molecular docking analysis of curcumin, green dashed lines represents hydrogen bond and its length. (A) Molecular docking of curcumin with CSF1R. (B) Molecular docking of curcumin with AR. (C) Molecular docking of curcumin with BACE1. (D) Molecular docking of curcumin with HMOX1. (E) Molecular docking of curcumin with AGER. (F) Molecular docking of curcumin with GSK3B. (G) Molecular docking of curcumin with IL1B. (H) Molecular docking of curcumin with STAT3. (I) Molecular docking of curcumin with NFKB1.

Supervision: Gang Ye.
Validation: Huaqiao Tang.
Writing—original draft: Xinyan Wu.
Writing—review & editing: Xinyan Wu, Gang Ye.

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