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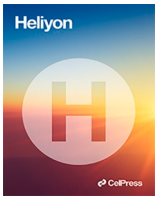

Research article

Exploring resveratrol against Alzheimer's disease and Parkinson's disease through integrating network pharmacology, bioinformatics, and experimental validation strategy in vitro

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ABSTRACT

Background: The study aims to investigate the pharmacological basis and molecular mechanisms of resveratrol in the treatment of Alzheimer's disease (AD) and Parkinson's disease (PD) through the approach of treating different diseases with the same method, guided by traditional Chinese medicine theory. Utilizing network pharmacology and bioinformatics methods, this research aims to provide modern medical evidence for the theory of treating different diseases with the same method in traditional Chinese medicine.

Methods: Omnibus from Swiss Target Prediction, TCMSP, SuperPred, SEA, HIT, CTD, TCMIP and Gene Expression Disease datasets for resveratrol related genes, Alzheimer's disease, and Parkinson's disease were obtained from the GEO database. Core targets were identified by weighted gene coexpression network analysis (WGCNA) and minimum absolute contraction and selection operator (LASSO). The expression of core targets was verified in AD and PD cell models. The immune characteristics of AD and PD were analyzed by CIBERSORT algorithm. Finally, the potential mechanism of resveratrol intervention on the core target was studied by molecular docking technique.

Results: The results of network pharmacological analysis showed that resveratrol acted on 85 common targets such as STAT3 and CASP3, affected AGE-RAGE signaling pathway and PI3K-Akt signaling pathway, and showed the effect of "same disease and different treatment" for AD and PD. Three core targets associated with AD and PD (PLK4, FCGRT, and PRKAR2A) were finally identified through comprehensive transcriptome analysis, and experimentally verified in cell models of AD and PD. At the same time, the analysis of immune cell infiltration suggested that AD and PD had dysregulation of inflammation, and the core target was significantly related to M2 macrophages.

Conclusion: Resveratrol may play a potential mechanism of "treating the same disease with different diseases" and target three core targets (PLK4, FCGRT and PRKAR2A) to improve the disease process of AD and PD by participating in the regulation of immune and inflammatory pathways. These findings have potential implications for clinical practice and future research.

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All the authors participated in this study and agreed to publish the paper.

1. Introduction

Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most prevalent neurodegenerative disorders, traditionally associated with "dementia" and "tremors," respectively, in Chinese medicine. AD is primarily characterized by patients losing familiarity with objects that were once known to them, along with a spectrum of cognitive deficits and behavioral limitations [1,2]. As the second most common neurodegenerative condition following AD, PD manifests through distinctive motor symptoms, including altered gait patterns and resting tremors [3,4]. The etiologies of AD and PD are complex and profoundly affect the quality of life. Recent robust clinical research into the etiologies of these diseases, particularly AD, has concentrated on neuronal apoptosis, hyperphosphorylation of tau protein, and the accumulation of senile plaques due to amyloid-beta (Aβ) deposition [5]. On the other hand, PD's etiology predominantly involves the deterioration and death of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies [6]. Nonetheless, the intricate etiologies of AD and PD result in a spectrum of detriments to patients' health, underscoring the importance of investigating shared pathogenic pathways to develop effective treatments, prevention strategies, and disease management.

Resveratrol, a polyphenolic compound, is recognized for its robust antioxidant, anti-inflammatory, and free radical scavenging effects. Studies have demonstrated that resveratrol can mitigate endothelial injury induced by atherosclerosis via the Pin1/Notch1 pathway [7], suppress the proliferation of the non-pathogenic bacterium Staphylococcus aureus, and ameliorate liver fibrosis in Balb/c mice [8]. In addition, resveratrol is posited to enhance the prognosis of AD [9]. During a randomized double-blind trial, patients receiving resveratrol exhibited no significant alterations in aβ40 concentrations within their blood and cerebrospinal fluid in comparison to the control group. Interestingly, at the study's conclusion, the placebo cohort displayed a notable decline in aβ40 levels, suggesting a possible protective role of resveratrol against Alzheimer's disease. Furthermore, in a PD rat model, resveratrol was found to reduce caspase-3 and xanthine oxidase activities, decrease IL-1β levels, shield neurons against apoptosis prompted by endoplasmic reticulum stress, re-establish dopamine concentrations, and markedly suppress the expression of CCAAT/enhancer-binding protein homologous protein and glucose-regulated protein through the activation of the glutathione peroxidase and Nrf2 signaling pathways [10]. Nonetheless, the research scope concerning the potential of resveratrol in combating Alzheimer's and Parkinson's diseases

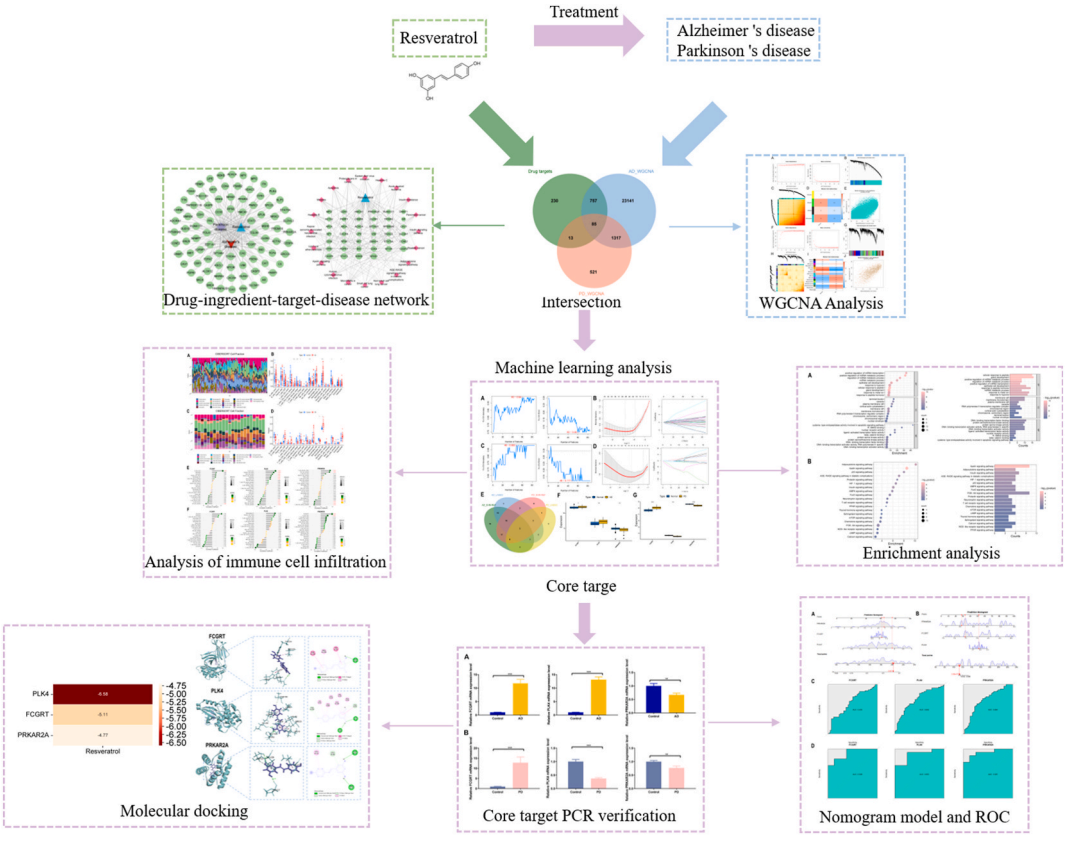

Fig. 1. Flow chart of this study.

remains limited.

The exploration of resveratrol's efficacy in AD and PD treatment has gained prominence in prior research. Nevertheless, these investigations have encountered challenges such as limited sample sizes, low study quality, an imbalance between clinical or theoretical focus and the lack of comprehensive mechanistic studies, as well as an insufficient consideration of the AD-PD interaction. Confronted with these issues, there is a compelling need for an extensive examination of resveratrol's underpinning effects and molecular mechanisms. Network pharmacology, which employs a system-wide approach alongside a biological network perspective to discern the intricate molecular interconnections between pharmaceuticals and pathologies, has emerged as a formidable instrument for elucidating the active constituents of traditional Chinese medicine, including their expansive mechanism of action. Additionally, whole-genome transcriptome analysis, harnessed through microarray technology and cutting-edge bioinformatics, is pivotal in pinpointing vital disease-related targets while ushering novel insights into the molecular mechanisms and classifications of diseases [11, 12]. This inquiry leverages network pharmacology techniques to investigate resveratrol's potential mechanisms in combating AD and PD, paired with bioinformatics to unearth the genetic interrelations between these diseases and resveratrol at the transcriptome tier and the additional interactions with immune cells. Moreover, this study aspires to corroborate core genes via co-expression analysis, sophisticated machine learning, molecular docking, and cellular experimentation, further deepening our grasp of resveratrol's impact on AD and PD pathophysiology. This could potentially inaugurate fresh research avenues for clinical intervention. The methodology employed in this study is depicted in Fig. 1.

2. Materials and methods

2.1. Drug target acquisition

The active compound of resveratrol was retrieved from the PubChem database in the form of standard SMILES structures. These SMILES structures were then cross-referenced in the Swiss Target Prediction, TCMSP, SuperPred, SEA, HIT, CTD, and TCMIP databases to identify the UniProtID corresponding to the target proteins of the specific compounds. Subsequently, the UniProtID information was transformed into Gene symbols utilizing the ID mapping utility within the Uniport database. Duplicate entries were eliminated to isolate the resveratrol-associated targets. To conclude, data from the Swiss Target Prediction, TCMSP, SuperPred, SEA, HIT, CTD, and TCMIP databases were consolidated, and redundant information was excluded to ascertain the drug targets relevant to resveratrol for the purposes of this research. Details of the database information are shown in Table 1.

2.2. Disease target acquisition

In the quest for disease targets, we explored Alzheimer's disease (AD) and Parkinson's disease (PD) datasets within the Gene Expression Omnibus (GEO) using the specified keywords. The AD dataset GSE122063 [13] served as the foundational training dataset, structured on the GPL16699 [Agilent-039494 SurePrint G3 Human GE v2 8 × 60K Microarray 039381 (Feature Number version)] platform, encompassing 56 AD patient samples and 44 healthy control samples. Analogously, the PD dataset GSE49036 [14] was curated on the GPL570 {[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array} platform, embracing 8 PD patient samples and 15 healthy control samples. All data utilized in the study were sourced from GEO, obviating the need for ethical clearance and informed consent.

2.3. Differential analysis of AD_PD disease and weighted gene Co-expression Network Analysis (WGCNA)

The dataset was normalized and analyzed using the R software, with the criteria of $|\log2FC| \geq 0.585$ and adj.P < 0.05 applied for the selection of differentially expressed genes (DEGs). Subsequently, the "WGCNA" R package was utilized to conduct weighted gene co-expression network analysis (WGCNA) for the identification of co-expression modules. The top 25 % of genes exhibiting the largest differences were chosen for the WGCNA analysis to ensure result accuracy. An optimal soft threshold was selected for constructing a weighted adjacency matrix, which was then converted into a topological overlap matrix (TOM). By setting the minimum module size to 50, modules were generated using the TOM-based dissimilarity measure (1-TOM) and hierarchical clustering tree algorithm. Each

Table 1
Related databases.

Database	URL
PubChem	https://pubchem.ncbi.nlm.nih.gov/
Swiss Target Prediction	http://www.swisstargetprediction.ch/
TCMSP	https://old.tcmsp-e.com/tcmsp.php
SuperPred	https://prediction.charite.de/subpages/target_prediction.php
SEA	http://bkslab.org
HIT	http://www.badd-cao.net:2345/
CTD	https://ctdbase.org/
TCMIP	http://www.tcmip.cn/TCMIP/index.php/Home/
GEO	http://www.ncbi.nlms.nih.gov/geo
String	https://cn.string-db.org/

module was allocated a random color, with the module eigengene representing the overall gene expression profile of that specific module. The association between modules and disease status was evidenced by module significance (MS), while gene significance (GS) was defined as the correlation between a gene and clinical phenotype.

2.4. Target selection for treatment

To elucidate the potential interaction between the WGCNA analysis results in patients with AD_PD and the principal active components associated with resveratrol targets, we will compute the intersection of AD_PD_WGCNA with the primary resveratrol-related targets. This analysis will be visually represented using the "Venn" package in the R software to generate a Venn diagram.

2.5. Construction of protein-protein interaction (PPI) network

The String database is an online bioinformatics repository aimed at offering insights into gene and protein interactions. The intersection targets are imported into the String database with a filter setting of "minimum required interaction score $\geq$0.15," followed by the download and storage of the PPI network graph and TSV format file. Subsequently, the PPI network is visualized using Cytoscape software (version 3.9.0) to create a multi-dimensional network focusing on the interaction between resveratrol and AD_PD.

2.6. Enrichment analysis of GO functions and KEGG pathways

Use R software to conduct GO and KEGG enrichment analysis on the intersecting targets, explore the potential biological functions and major signaling pathways of the resveratrol treatment for AD_PD diseases. Sort in descending order based on p-value, using q value < 0.05 as the threshold, select significantly different enrichment results, and output the biological functions of enriched GO terms and the top 20 KEGG signaling pathways.

2.7. Core gene screening

In this research, we utilized two machine learning algorithms, namely LASSO and SVM-RFE, to identify core genes within the intersecting targets. The LASSO method and SVM-RFE were implemented for distinguishing diagnostic biomarkers of AD_PD. We performed a 10-fold cross-validation with the glmnet package to discriminate between AD_PD patients and healthy controls. To mitigate overfitting, the SVM-RFE algorithm was utilized for core gene selection employing the e1071 and svmRadial packages. Finally, core gene expression analysis was conducted using the "corrplot" and "ggplot2" functions in the R software.

2.8. Immunocyte infiltration analysis

The CIBERSORT algorithm (http://cibersortx.stanford.edu) was employed to ascertain the relative proportions of 22 infiltrating immune cell types in each tissue. Immunological scores for individual samples were derived through the use of the "ESTIMATE" algorithm. Additionally, the correlation between core targets and the quantity of infiltrating immune cells was assessed through Spearman rank correlation analysis in the R software.

2.9. Molecular docking

The study involved the extraction of core target-related protein structures in PDB format for intersection target screening from the RCSB database, as well as obtaining the structure of the resveratrol compound from the PubChem database. The target proteins and small molecule compounds were preprocessed using AutoDock software, followed by conversion and analysis of their protein binding sites to identify relevant active docking pockets. Subsequently, the target proteins and small molecule compounds were imported into the AutoDock Vina 1.1.2 docking software, where the docking site coordinates were set, docking verification was conducted, and the docking results were visualized using PyMOL software.

Table 2
Primer sequences used for quantitative Real-time PCR (qRT-PCR).

Disease	Gene	Forward(5'→3')	Reverse(5'→3')
AD	GAPDH	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
AD	FCGRT	ATCATCTCACGGCTGTGTCAA	AGGTCAGATACTGCTGAGGAC
AD	PLK4	GAGAGGATCGAGGACTTTAAGGT	TGGACTCAGCTCTGTAGACAC
AD	PRKAR2A	GGAGGATAACGATCCAAGGGT	TGCTCGTCAGTTTTGACAATCT
PD	GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC
PD	FCGRT	GGGGAAAAGGTCCCTACACTC	CCTGCTTGAGGTCGAAATTCAT
PD	PLK4	AAGCTCGACACTTCATGCACC	GCATTTTCAGTTGAGTTGCCAG
PD	PRKAR2A	GTGCTGAGACCTATAACCCTGA	ACATGGCATCGAGAACTTGAGA

2.10. Reverse transcription-quantitative polymerase chain reaction (RT-PCR) analysis

qRT-PCRRNA extraction and qRT-PCR procedures were performed according to the above methods [15,16]. The corresponding mRNA levels were calculated using the 2ˆ(-DDCt) formula. Primers used in this study are summarized in Table 2.

2.11. Statistical analysis

All statistical analyses were performed using R software version 4.1.0. To assess the statistical significance between normally distributed variables in two sets of continuous variables, we used an independent student ST test. Instead, the Mann-Whitney *U* test is used to determine the differences between the non-normally distributed variables. Chi-square test or Fisher exact test were used to analyze the statistical significance between the two groups of categorical variables. The correlation coefficients between different

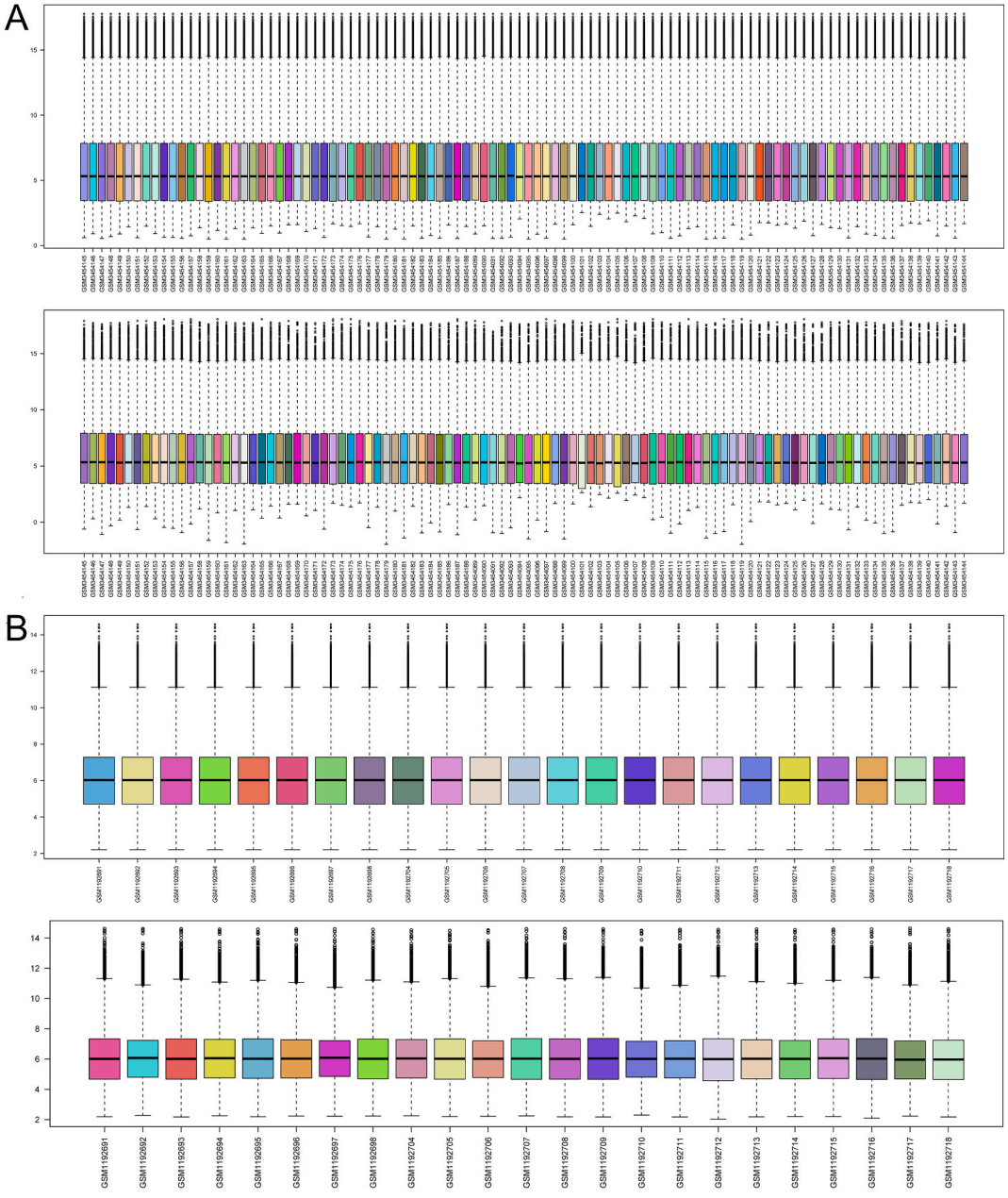

Fig. 2. Normalization Processing Chart (A) Bar graphs before and after standardization in AD samples; (B) Histogram before and after standardization in PD samples.

genes were estimated by Pearson correlation analysis. All statistical tests were double-sided, with the level of statistical significance set at a P-value of less than 0.05.

3. Results

3.1. Acquisition of resveratrol drug targets and targets for AD_PD disease

Through the Swiss Target Prediction, TCMSP, SuperPred, SEA, HIT, CTD, and TCMIP databases, we identified 69, 151, 110, 56, 224, 991, and 25 resveratrol-related targets, respectively. After eliminating duplicates, a total of 1085 unique targets associated with resveratrol were retained. Subsequently, we conducted an analysis of data related to AD_PD disease. Initially, in order to mitigate batch effects across samples, the dataset underwent normalization prior to the differential analysis. The figures presented data before (Fig. 2A) and after (Fig. 2B) normalization. Differential analysis on the AD_PD dataset was then carried out using the "limma" package in R software. The analysis yielded a total of 3459 differentially expressed genes (DEGs) from the AD dataset, comprising 1573 upregulated targets and 1886 downregulated targets. Similarly, from the PD dataset, 51 DEGs were identified, with 17 upregulated targets and 34 downregulated targets. Additionally, volcano plots and heat maps were created (Fig. 3A–B).

3.2. Acquisition of AD_PD related targets

In order to identify key gene modules associated with AD_PD, we utilized the WGCNA algorithm to build co-expression networks and modules for both control subjects and individuals with AD_PD. The variance of gene expression was computed in the AD-related dataset GSE122063 and the PD-related dataset GSE49036, followed by the selection of the top 25 % of genes with the highest variances for further investigation. By applying soft thresholding power values of 2 and 7, and attaining a scale-free R2 value of 0.9, co-expressed

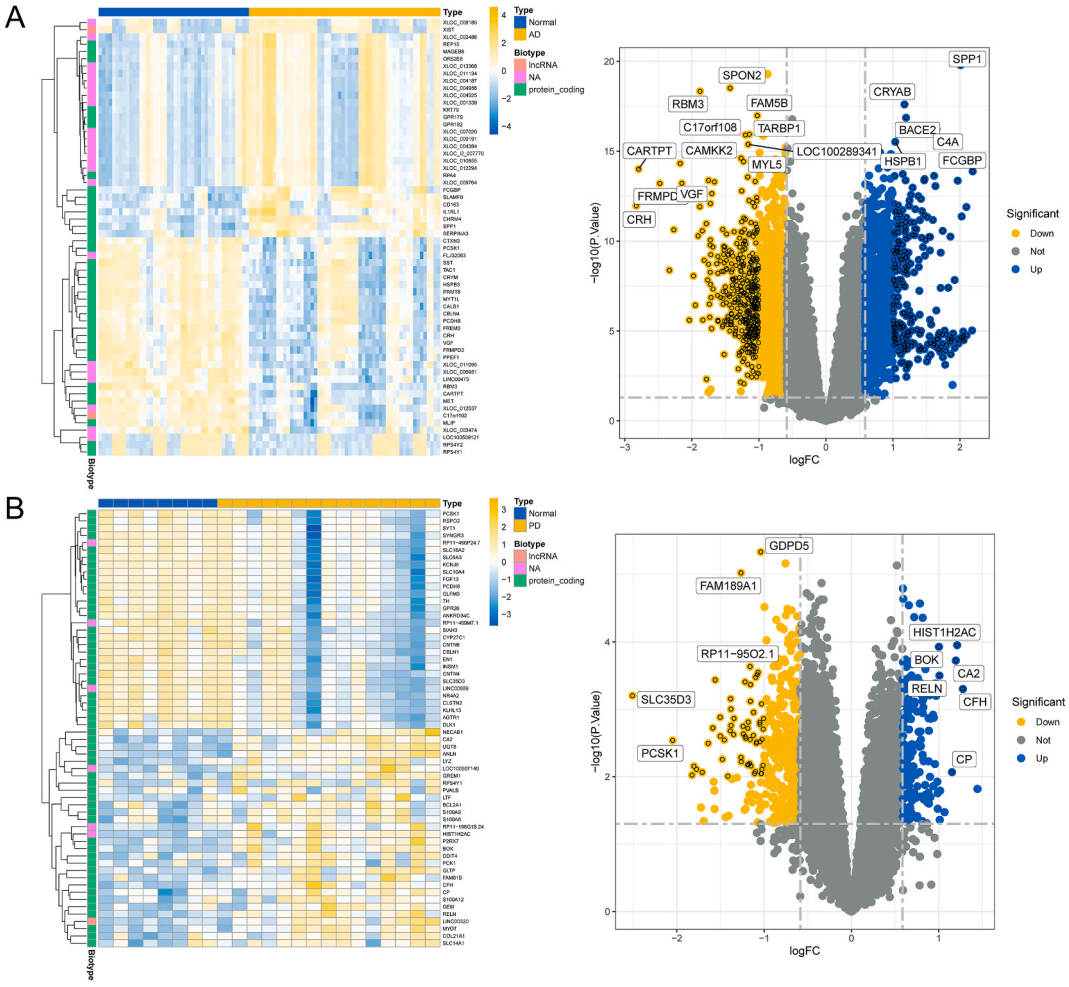

Fig. 3. Differential Analysis Heatmap and Volcano Plot (A) Differential gene heat maps and volcano maps in AD samples; (B) Differential gene heat maps and volcano maps in PD samples.

gene modules were successfully delineated (Fig. 4A–F). Through the dynamic tree cutting algorithm, distinct co-expression modules were identified, with 4 modules in AD and 16 in PD, each represented by different colors, and the topological overlap matrix (TOM) heatmap was illustrated (Fig. 4B–C, G-H). Subsequent analysis focused on exploring co-expression patterns and relationships in the 4/16 colored modules of the AD_PD dataset concerning clinical characteristics (normal controls vs. AD_PD group). Notably, the turquoise module in AD and the brown module in PD exhibited the most robust association in AD_PD, encompassing 25,300 and 1936 genes, respectively (depicted in Fig. 4D–I). Additionally, a negative correlation was identified between the turquoise module in AD and the brown module in PD across diverse AD_PD samples (Fig. 4E–J).

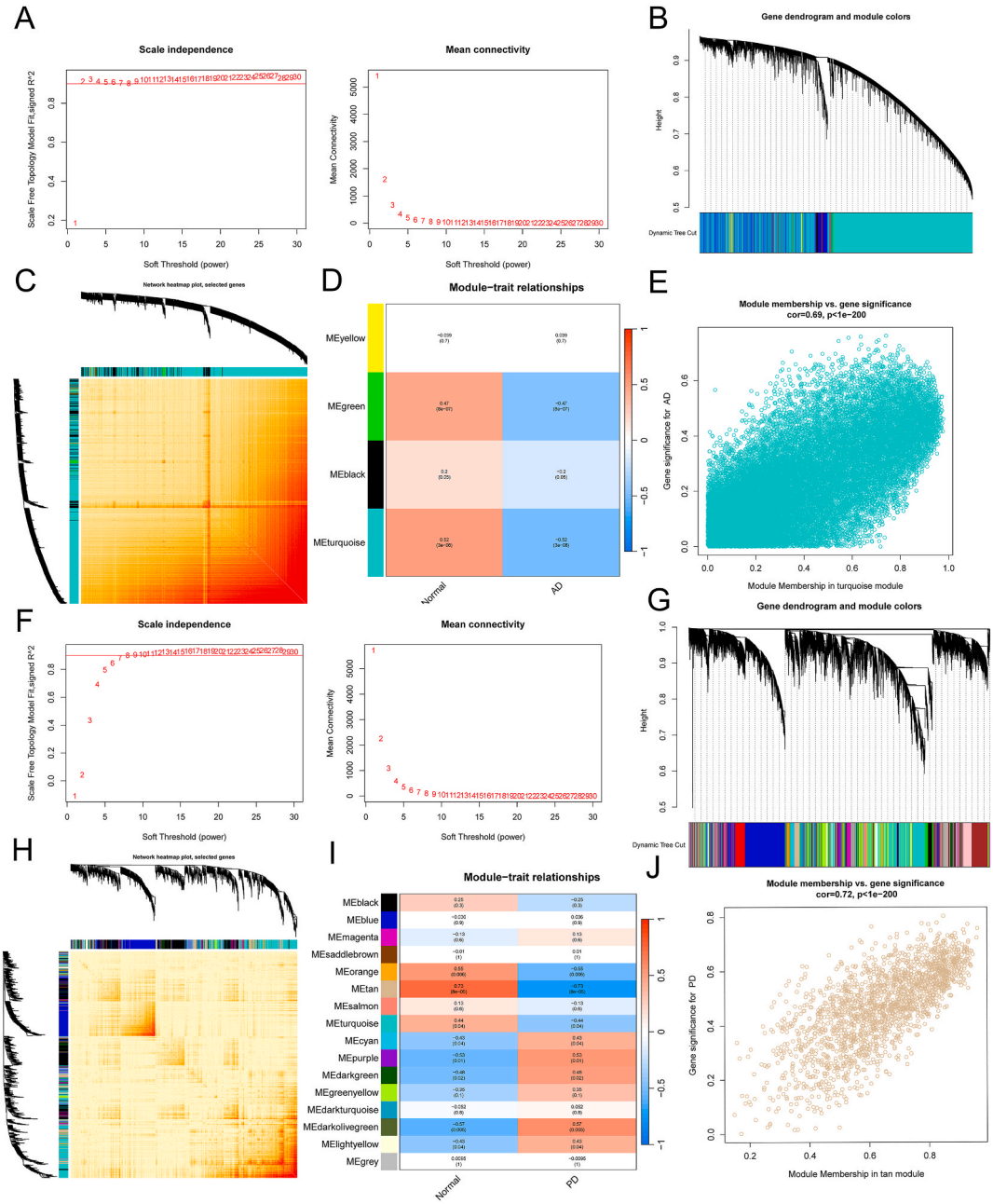

Fig. 4. WGCNA analysis graph (A,F) Soft threshold power selection. (B,G) The clustering tree diagram of the co-expression module. Different colors represent different co-expression modules. (C,H) Representative heat maps of correlations between modules. (D,I) Correlation analysis between module characteristic genes and clinical status. Each row represents a module; Each column represents a clinical state. (E,J) Scatter plots of module membership and AD_PD gene significance in turquoise and brown modules.

3.3. Intersection target screening and drug component target network construction

To investigate the potential mechanism of resveratrol in treating AD_PD, we analyzed the intersection of resveratrol drug targets with differential genes in AD_PD and WGCNA-related targets, identifying a total of 85 intersecting genes (Fig. 5A). Subsequently, we constructed the "resveratrol-AD_PD disease" network using Cytoscape software (version 3.9.0) to further elucidate the potential association between resveratrol-related targets and AD_PD disease. Upon importing the prepared "Network.xlsx" file into Cytoscape software, the "resveratrol-AD_PD disease" network was generated (Fig. 5B), consisting of 88 nodes (comprising 1 active ingredient node, 50 target nodes, and 1 disease node) and 255 edges. The topological parameters of the network were analyzed using the Network Analyzer plugin, revealing an average neighbor count of 5.795, network heterogeneity of 2.568, network density of 0.067, and network centrality of 0.0932. Each edge illustrates the interaction between resveratrol and its target, as well as the relationship between AD_PD disease and the target. Resveratrol demonstrates the potential to interact with multiple targets effectively, serving as a central hub in the network for targeting AD_PD disease. Consequently, the therapeutic strategy of resveratrol in addressing AD_PD involves modulating multiple targets collectively.

3.4. Construction and enrichment analysis of intersection genes PPI network

In order to enhance our comprehension of the mechanism by which resveratrol functions in treating AD_PD disease, we utilized the PPI network to analyze the interactions among its intersecting targets. 85 intersecting targets associated with AD_PD disease were inputted into the String database to elucidate the interaction relationships among the component targets, resulting in 85 proteins and 1220 interaction lines. The intensity of color represents a higher Degree value, while the size of the node indicates the significance of the protein within the network. The visualization of the drug-disease intersection effects and target protein interactions (PPI) network graph is depicted in Fig. 5C. To pinpoint the core targets crucial to the efficacy of resveratrol in treating AD_PD disease, we identified the top twenty targets ranked by Degree value as core targets, detailed in Table 3. These 20 targets are pivotal players in the onset and progression of resveratrol's therapeutic impact on AD_PD disease.

3.5. Enrichment analysis

From the conducted KEGG and GO enrichment analyses, we have identified key biological processes that shed light on the functions of target proteins. Utilizing R software, we analyzed 85 overlapping targets associated with AD_PD to explore their roles in gene function and signaling pathways. The GO enrichment analysis conducted with the "Cluster Profiler" R software package revealed 901 entries, where 843 were linked to biological processes (BP), focusing on cellular response to peptides, gland development, and positive regulation of miRNA metabolic processes. Furthermore, we identified 36 entries in molecular function (MF), such as DNA-binding transcription factor binding and various protein kinase activities, along with 22 entries in cellular components (CC), primarily associated with membrane properties. Notably, our findings suggest the involvement of potential targets in biological processes like inflammatory responses and cell cycle regulation, which are closely tied to the onset of AD_PD. This underscores the multifaceted therapeutic effects of resveratrol on AD_PD through its impact on diverse biological processes (Fig. 6A).

Using the "Cluster Profiler" package in R software, we conducted KEGG pathway enrichment analysis on the 85 intersecting targets of resveratrol in AD_PD disease, which identified a total of 85 significantly enriched signaling pathways influenced by resveratrol intervention in AD_PD disease (q value < 0.05). These pathways mainly include the Apelin signaling pathway, Adipocytokine signaling pathway, AGE-RAGE signaling pathway in diabetic complications, and PI3K-Akt signaling pathway. The enrichment results were presented in Fig. 6B. The network representing the "Active ingredient-Target-Pathway" relationship (Fig. 7) consisted of 61 nodes and 204 edges. Utilizing the Network Analyzer plugin, we analyzed the network's topological parameters, revealing an average number of

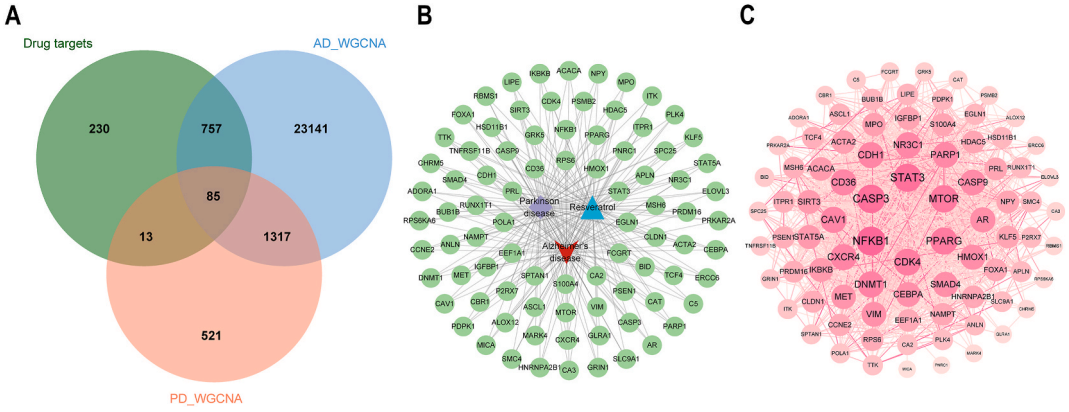

Fig. 5. Shared target genes of resveratrol in Alzheimer's disease and Parkinson's disease (A) Intersection diagram of resveratrol drug targets with AD_WGCNA and PD_WGCNA; (B) Drug - Ingredient - Target - disease map; (C) PPI network diagram.

Table 3
Top 20 core target genes ranked by Degree value.

Gene	Degree	BetweennessCentrality	ClosenessCentrality
NFKB1	70	0.066986	0.857143
CASP3	66	0.04211	0.823529
STAT3	65	0.059573	0.815534
MTOR	60	0.038215	0.777778
PPARG	59	0.034731	0.770642
CDK4	57	0.033357	0.756757
DNMT1	54	0.020074	0.736842
CAV1	53	0.027736	0.730435
CXCR4	53	0.03913	0.730435
CDH1	52	0.012068	0.724138
CD36	52	0.019741	0.717949
NR3C1	51	0.017937	0.717949
PARP1	50	0.015038	0.711864
CASP9	49	0.010099	0.7
HMOX1	47	0.012028	0.694215
SMAD4	47	0.016014	0.694215
CEBPA	45	0.009444	0.682927
VIM	45	0.015651	0.682927
ACACA	38	0.012224	0.636364
PRL	33	0.009681	0.622222

adjacent nodes of 6.689, network heterogeneity of 0.887, network density of 0.111, and network centrality of 0.574. Our analysis demonstrates that the targets of resveratrol's active ingredients are intricately linked to multiple pathways, showcasing a multi-target, multi-pathway effect crucial for the therapeutic efficacy in treating AD_PD disease, culminating in a complex interaction characterized by interconnectedness, coactivity, and multi-level regulation.

3.6. Core target selection

In this study, we sought to assess the diagnostic potential of intersecting targets in AD_PD patients compared to healthy populations. Utilizing the AD_PD dataset, we employed two machine learning algorithms, namely LASSO and SVM-RFE, to identify significant core

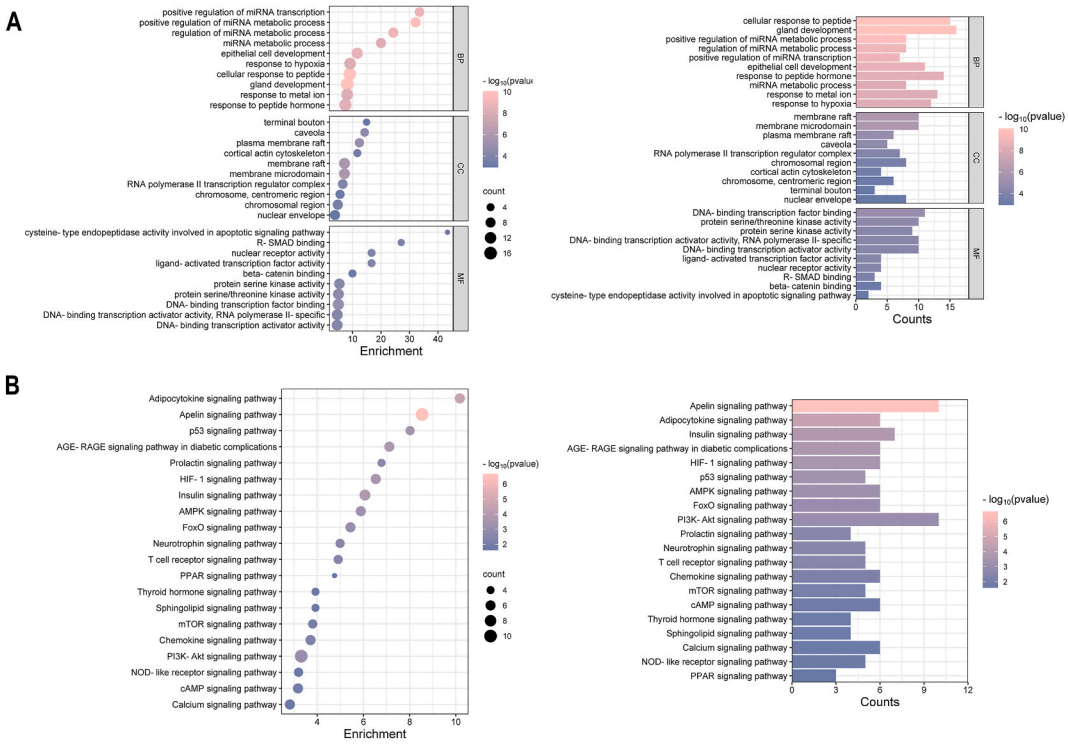

Fig. 6. Enrichment analysis diagram (A) GO and KEGG enrichment analysis in AD samples; (B) GO and KEGG enrichment analysis in PD samples.

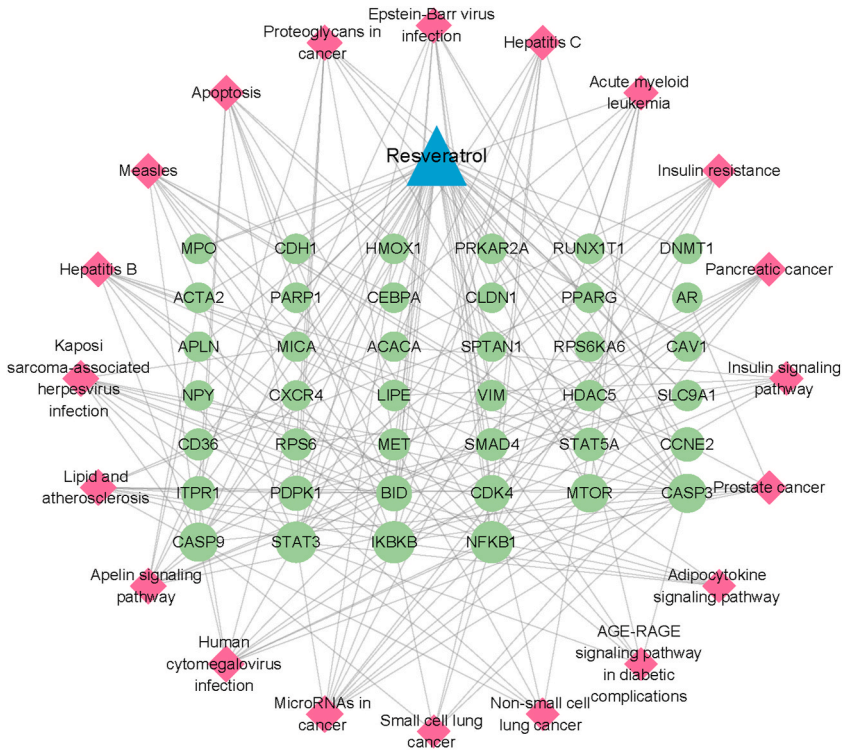

Fig. 7. Active ingredient-target-pathway network diagram.

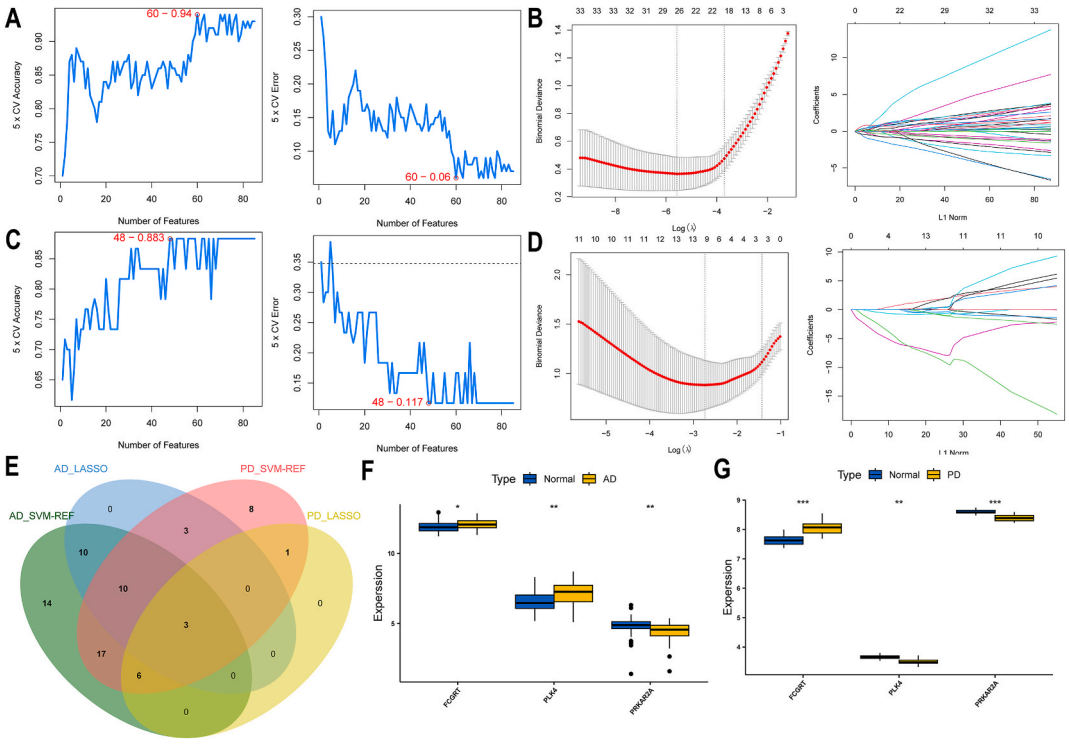

Fig. 8. Identification of final core targets through machine learning methods. (A,C) SVM-REF analysis diagram; (B,D) lasso regression analysis diagram; (E) Intersection of three machine learning outcomes.

targets for discriminating AD_PD patients. In the AD sample, the SVM-RFE algorithm revealed 60 genes out of the 85 intersecting targets as core targets (Fig. 8A), while the LASSO algorithm selected 26 genes from the same set (Fig. 8B). Analyzing the PD sample, we identified 48 core target genes using the SVM-RFE algorithm (Figs. 8C) and 10 core target genes using the LASSO algorithm (Fig. 8D). Integrating the outcomes of both algorithms, we pinpointed three genes, namely PLK4, FCGRT, and PRKAR2A, as core targets (Fig. 8E). Subsequently, we conducted expression analyses of these core targets in AD and PD samples, detailed in Fig. 8F–G. Notably, the expression of FCGRT was markedly reduced in AD_PD patients, while that of PRKAR2A exhibited a significant rise. Intriguingly, the expression of PLK4 displayed a negative correlation between AD and PD patients (Fig. 8F–G). RT-qPCR results confirmed that the mRNA level of PRKAR2A was increased in AD _ PD samples, and the mRNA level of FCGRT was decreased in AD _ PD samples (Fig. 9). Finally, in order to elucidate the diagnostic potential of key genes in distinguishing AD_PD samples from healthy control samples, we conducted the construction of the neographic model and ROC curve analysis of key genes. The results of the nomogram model showed that the risk rate of Resveratrol's intervention to treat key genes in the development of AD_PD was predicted by calculating the sum of the expression scores of these key genes to determine treatment sensitivity based on the individual score scale drawn according to key genes (Fig. 10A–B). Furthermore, the bar plot and ROC curve analyses demonstrated that the AUC values of the identified core targets, namely PLK4, FCGRT, and PRKAR2A, exceeded 0.65, underscoring their high diagnostic accuracy (Fig. 10C–D).

3.7. Analysis of immune cell infiltration

The pathogenesis of AD_PD is intricate, with the immune system playing a crucial role [17,18]. To investigate differences in the immune microenvironment between AD_PD patients and healthy controls, we employed the CIBERSORT algorithm. In AD samples (Fig. 11A–B), T cells (CD4 memory activated), Macrophages M2, and Neutrophils exhibited significant elevation, while T cells (follicular helper), T cells (regulatory - Tregs), NK cells (activated), and Mast cells (resting) showed notable decreases. Meanwhile, in PD samples (Fig. 11C–D), Macrophages M2 significantly increased, contrasting with the decrease in Macrophages M1. Noteworthy is the significant elevation of Macrophages M2 in both AD and PD samples. Furthermore, we analyzed the relationship between core target expression levels and the immune microenvironment. Results indicated that in AD samples, B cells (naive) positively correlated with FCGRT and PLK4, while being negatively correlated with PRKAR2A. Contrarily, in PD samples, PRKAR2A exhibited a significant positive correlation with B cells (naive). Eosinophils showed significant positive correlation with PLK4 and negative correlation with PRKAR2A (Fig. 11E–F). These findings imply a close association between changes in the immune microenvironment of AD_PD patients and core targets like FCGRT, PLK4, and PRKAR2A.

3.8. Molecular docking

The strength of interaction between target proteins and active ingredients is indicated by docking scores. A negative numerical value of the free binding of the active ingredient to the target implies stronger binding, with lower values indicating better binding. A

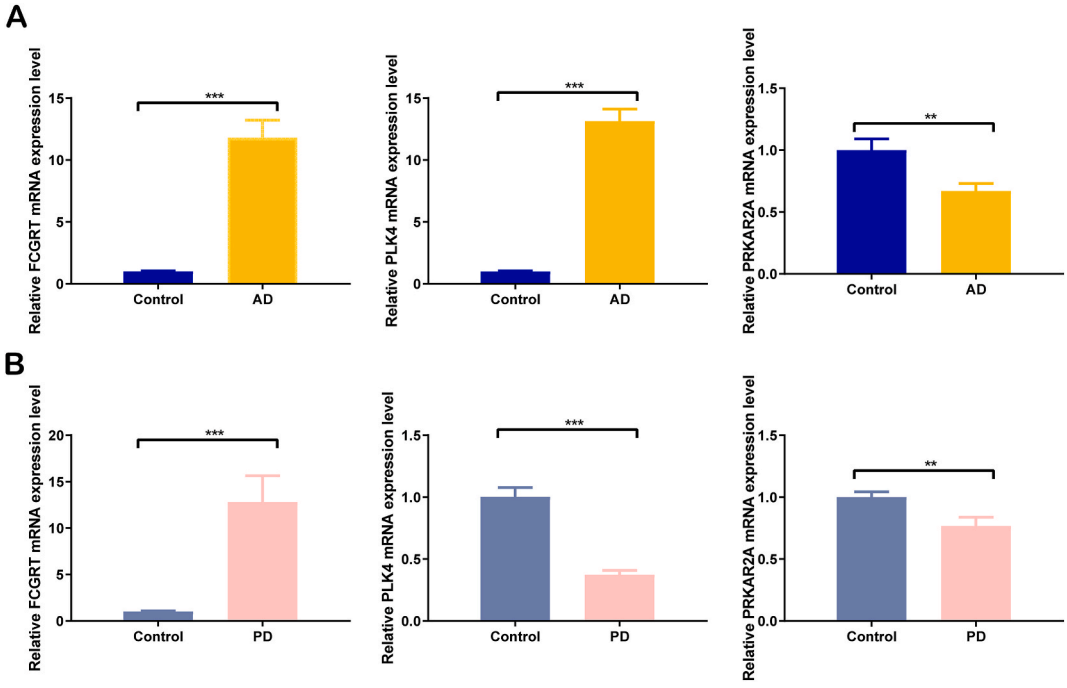

Fig. 9. RT-qPCR verification of core genes.

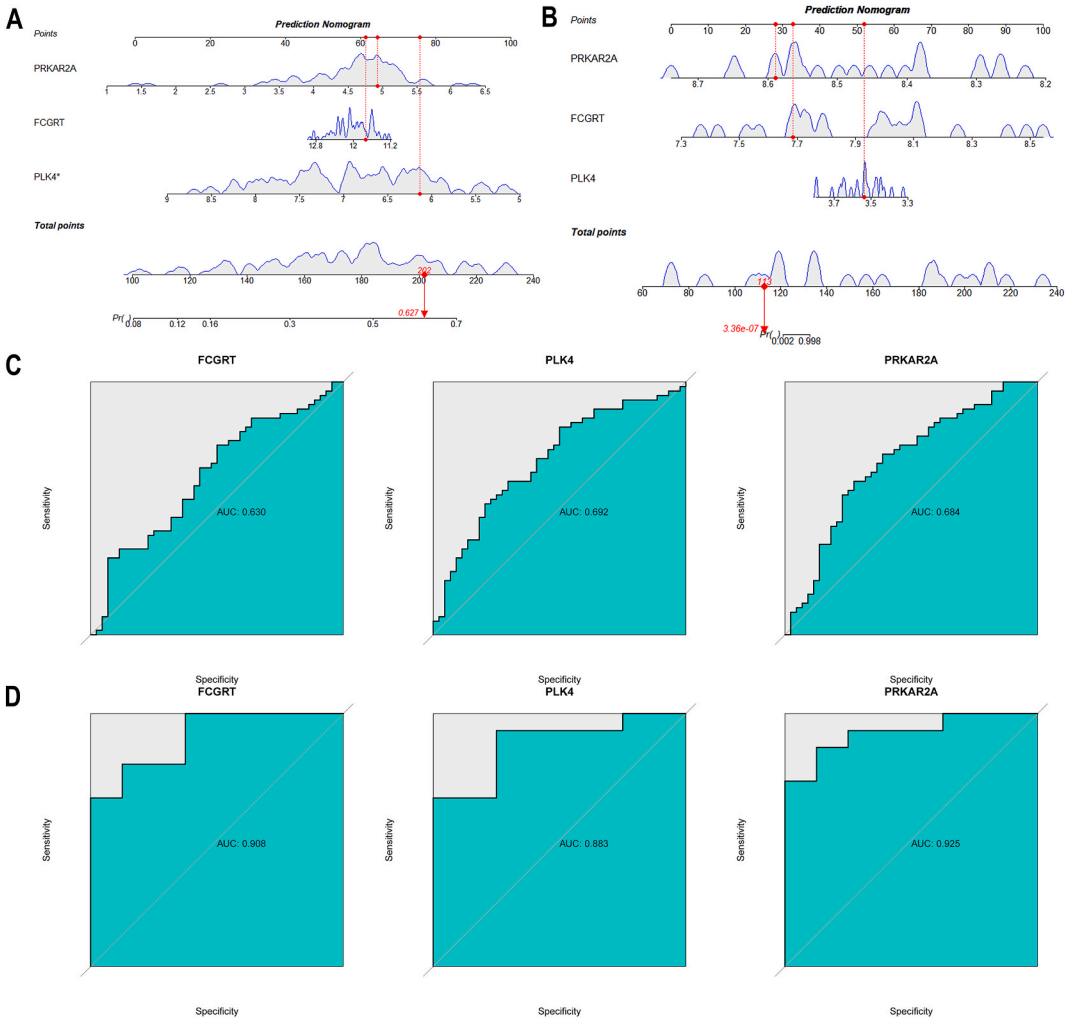

Fig. 10. Nomogram model of core targets and ROC curve analysis (A–B) Key gene nomogram model; (C–D) ROC curves of key genes in AD_PD samples.

binding energy of −5 kcal/mol suggests a favorable interaction between the receptor protein and the ligand small molecule, with a more robust binding activity observed when it is below −7 kcal/mol (see binding energy heat map in Fig. 12). In the FCGRT molecule docking results, the binding energy between the small molecule and the receptor is −5.11 kcal/mol, demonstrating a positive binding effect. The small molecule primarily interacts with the receptor protein through hydrogen bonding with GLN A:34. In the PLK4 molecule docking outcomes, the binding energy between the small molecule and the receptor is −6.58 kcal/mol, suggesting an effective binding affinity. The small molecule predominantly engages with the receptor protein through hydrogen bonding associated with CYS A:92 and GLU A:90. For the PRKAR2A molecule docking results, the binding energy between the small molecule and the receptor is −4.77 kcal/mol, indicating a good binding effect. The small molecule primarily interacts with the receptor protein through hydrogen bonding linked to ASP B:27 and PRO A:7 (Fig. 13).

4. Discussion

Recent years have witnessed a burgeoning interest in elucidating the pathological mechanisms underlying AD and PD, spanning various domains and supported by an extensive corpus of literature. This has solidified the groundwork for examining their shared pathogenic pathways. According to Western medicine, aging has been indisputably identified as the primary aetiological factor for both AD and PD [19]. Contrastingly, Traditional Chinese Medicine (TCM) conceptualizes aging as a gradual diminution of renal essence, which manifests as an initial rise followed by a subsequent fall. This renal essence insufficiency impairs the nourishment of the brain marrow, rendering it vacuous and susceptible to pathogenic invasions such as phlegm and turbidity. Specifically, AD is frequently associated with phlegm and stasis, whereas PD is characterized by these same pathogenic invasions along with symptoms of internal wind. In this context, targeting phlegm and stasis is fundamental in TCM for investigating their shared pathological traits. Our

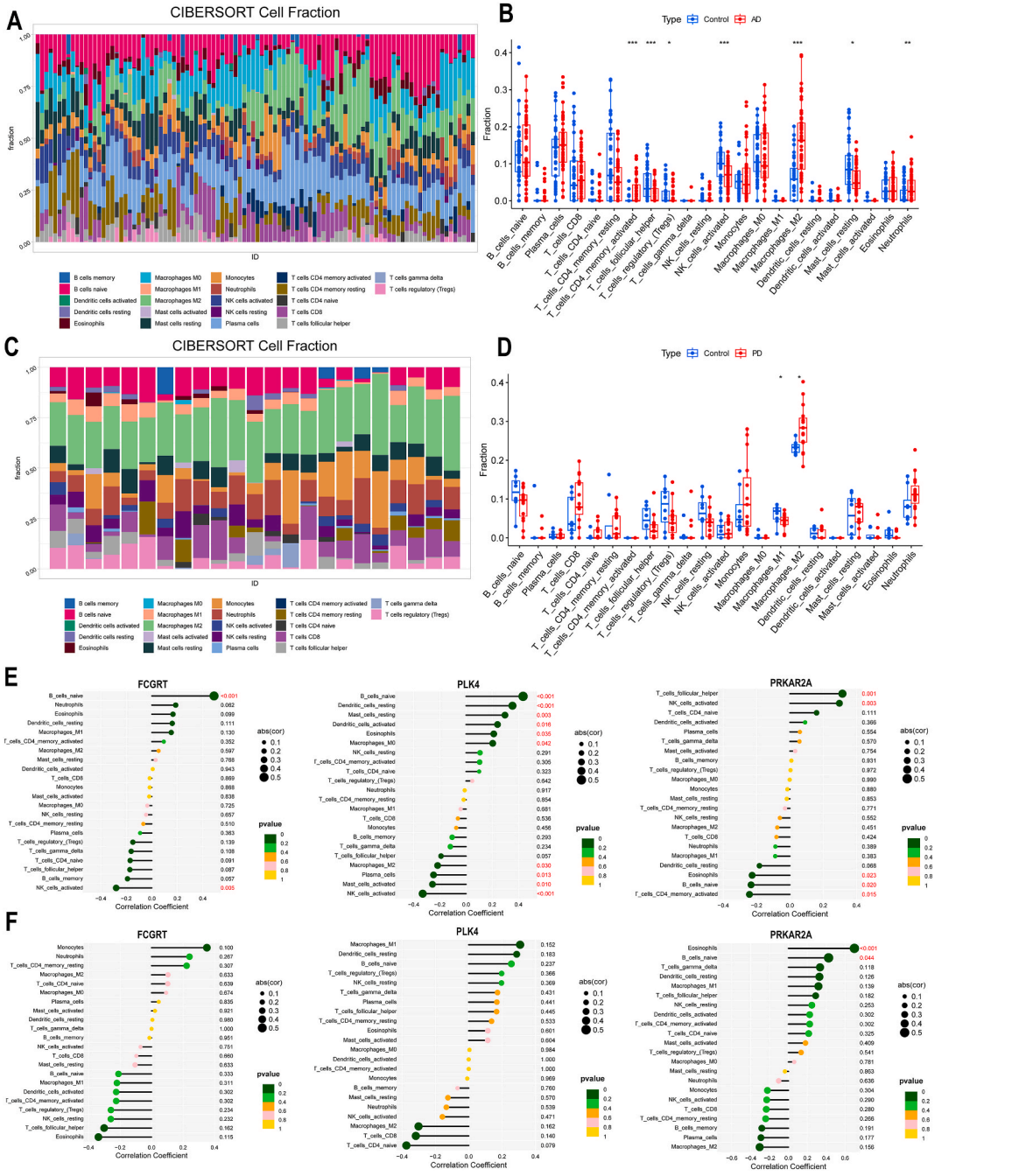

Fig. 11. Immune cell infiltration diagram (A,C) box diagram of immune cell fraction in AD_PD sample; (B,D) Bar chart of the relative percentage of each type of immune cell in the AD_PD sample; (E,F) Histogram of correlation between key genes and immune cells.

study endeavors to elucidate the molecular mechanisms through which resveratrol treats AD and PD, adopting an approach that uses a uniform treatment for distinct diseases. We aim to perform bioinformatics analyses on shared genetic markers to aid in developing novel therapeutic interventions. Data mining from databases like Swiss Target Prediction, TCMSP, SuperPred, SEA, HIT, CTD, and TCMIP resulted in the compilation of 1085 genes associated with resveratrol. Furthermore, we consulted the GEO database for AD and PD datasets, employing the WGCNA algorithm to generate a co-expression network that contrasts healthy individuals against AD-PD patients, identifying the cyan module (25300 genes) and the brown module (1936 genes) as having the most pronounced negative correlation amongst AD-PD sample variations. The integrative analysis of these modules discerned 85 genes common to the treatment of AD-PD with resveratrol.

In our mechanistic studies, the pathway network mapping of 85 intersection genes identified as part of our enrichment analysis is

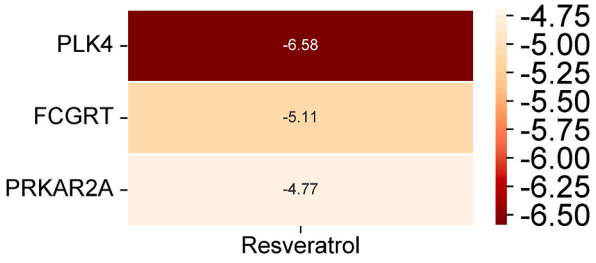

Fig. 12. Binding energy heatmap.

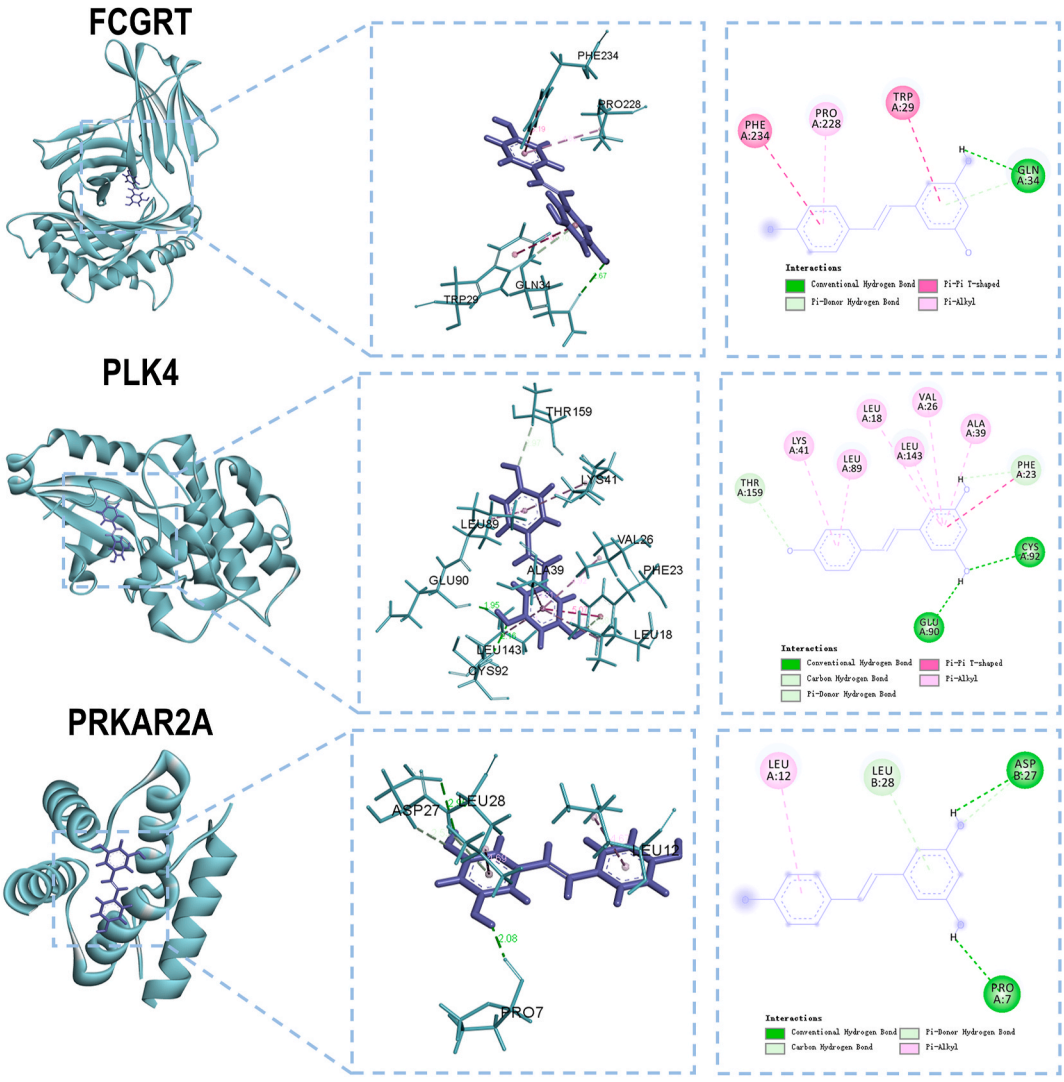

Fig. 13. Visualization of molecular docking.

pivotal. Key signaling pathways, such as the AGE-RAGE pathway associated with diabetic complications, HIF-1 pathway, and the PI3K-Akt pathway, were notably enriched. Type 2 diabetes exacerbates the progression of AD by increasing the accumulation of advanced glycation end products (AGEs) and their receptor (RAGE) under hyperglycemic conditions, which then catalyzes the generation of reactive oxygen species (ROS), culminating in irreversible neuronal damage and attrition [20,21]. Resveratrol potentially attenuates ROS production by disrupting the AGE-RAGE interaction within the brain, thereby mitigating synaptic damage and enhancing cognitive functions. The AGE-RAGE pathway also instigates ROS production, activates the transcription factor NF-κB leading to tissue

injury and inflammatory reactions [22,23], and plays a role in cellular damage pathways. Inhibition of the AGE-RAGE axis may thus alleviate oxidative stress in PD by tempering the NF-κB pathway activity [24]. Moreover, hypoxia-inducible factor-1 (HIF-1), comprising subunits HIF-1α and HIF-1β, is implicated in PD development, particularly via the upregulation of tyrosine hydroxylase (TH). HIF-1 elevation augments TH expression and dopamine (DA) synthesis and release in the substantia nigra of the midbrain [25]. Emerging evidence suggests the critical involvement of the PI3K/AKT pathway in the brain's oxidative and inflammatory responses [26], and along with the MAPK pathway, it governs cellular processes like growth, proliferation, and differentiation [27,28]. PI3K/AKT pathway, a key negative autophagy regulator, mediates neuroprotection in PD through miR-221 upregulation, which targets PTEN and influences the signaling cascade [27]. Collectively, these studies indicate that the aforementioned pathways are central to oxidative stress, inflammation, cellular harm, endothelial malfunction, DA synthesis, and other factors pivotal to AD and PD pathogenesis. Resveratrol's therapeutic or palliative prospects in AD and PD may stem from its capacity to modulate these pathways beneficially.

This study primarily investigates the nexus between AD and PD by discerning three biomarkers: PLK4, FCGRT, and PRKAR2A. The derived diagnostic model, incorporating these biomarkers, exhibits robust predictive capabilities for both disorders. PLK4 is a serine/threonine protein kinase characterized by its C-terminal polo-box domain [29], crucial for centriole duplication via phosphorylation [30]. Deviant expression patterns of PLK4 can precipitate genomic instability and contribute to tumorigenesis by promoting an aberrant increase in centriole numbers [31]. Furthermore, PLK4 is implicated in neuronal development and functionality; in the context of AD, it is linked to amyloid aggregation and potential synaptic dysfunction integral to the disease's pathogenesis [32]. Regarding PD, several studies have reported alterations in PLK4 expression levels, affirming its significance in the disease's progression [33]. FCGRT encodes for the alpha chain of the neonatal Fc receptor (FcRn), an IgG and albumin transporter, vital for maintaining their serum levels through pH-dependent mechanisms and mediating antigen uptake and immune complex presentation [34]. Recent evidence underscores FCGRT's critical function in the nervous system-its association with neuroinflammation, autoimmunity, and its possible role in modulating neuroinflammatory pathways in AD and PD [35]. PRKAR2A, which encodes a regulatory subunit of protein kinase A, occupies a fundamental niche in cell signaling [36]. Emerging research postulates that PRKAR2A expression alterations in individuals with AD and PD could correlate with neuronal functionality and survivability [37], denoting its prospective involvement in neurodegenerative conditions. Collectively, the three biomarkers identified propose a novel lens to enhance our comprehension of the molecular underpinnings of AD and PD.

The changes in immune cells appear to play an important role in the relationship between AD and PD. This study found significant infiltration of M2 macrophages in patients with AD and PD, and M2 macrophages, B cells were significantly associated with three biomarkers. M1 macrophages exhibit pro-inflammatory responses, while M2 macrophages are considered anti-inflammatory, involved in tissue repair and inflammation resolution [38,39]. A limited study found that transplantation of M2 macrophages could reduce neuronal loss in an AD mouse model, improve inflammatory response, and enhance cognitive function, suggesting a protective role of this subtype of macrophages in AD [40]. PD is a common incurable neurodegenerative disease. Anglade et al. [41] found that the progressive loss of dopaminergic neurons in the substantia nigra of PD is related to apoptosis of nigral neurons and macrophage autophagy. B cells are a major cell type in the immune system, playing important roles in immune regulation and defense [42]. Recent studies have suggested that B cells may be involved in excessive inflammatory responses and amyloid plaque formation, accelerating disease progression [43]. Some studies have also found abnormally increased B cells in the cerebrospinal fluid of AD patients, further supporting the potential role of B cells in disease onset [44]. In PD, B cells are also thought to potentially influence disease development [45]. Research indicates that B cells may selectively affect the peripheral immune system, alter inflammation levels, interact with T cells, thereby influencing the extent of neurodegeneration and inflammatory responses [46]. Overall, M2 macrophages and B cells demonstrate important roles and potential value in the study of AD and PD, while resveratrol may improve the disease progression in AD and PD patients by regulating the expression of PLK4, FCGRT, PRKAR2A, thus affecting M2 macrophages and B cells.

This investigation draws upon the principle of 'homotherapy for heteropathy' and assimilates multifaceted data encompassing "drug-disease-patient" dimensions. Employing advanced techniques that include network pharmacology, machine learning, molecular docking, and cellular experimentation, this study delves into the underlying mechanisms of resveratrol's therapeutic effects on AD and PD. It extends beyond examining the compound's safety and efficacy, instead focusing on elucidating the modes of action at genetic, proteomic, immunological, and signaling pathway levels. Contrary to preceding studies on traditional Chinese medicine or individual compounds that either utilized non-human subjects or relied solely on disease targets from GEO datasets, our research adopts a drug-centric approach to disease intervention. It meticulously analyses the complex roles of resveratrol by using targeted mechanisms as nexus points and substantiates findings with extensive dataset assessments, molecular modeling, and cellular validations. Hence, our comprehensive methodology surpasses previous endeavors, offering greater insights for clinical applications. Notwithstanding its thoroughness, the research presents limitations. Specifically, while we have discerned and corroborated the expression of three biomarkers through integrated network pharmacology and bioinformatics combined with cellular tests in AD and PD, their exact pathological functions warrant deeper exploration. Moreover, our findings predominantly derive from public repositories, devoid of intricate clinical nuances; thus, expanded clinical datasets are essential for a more robust correlation between these biomarkers and diverse clinical prognoses.

5. Conclusion

In summary, this study integrates the 'homotherapy for heteropathy' concept and employs network pharmacology, molecular docking, and bioinformatics approaches to elucidate the therapeutic targets and mechanisms of resveratrol in treating AD and PD. We have identified the critical pharmacological targets and signaling pathways, including three pivotal genes (PLK4, FCGRT, PRKAR2A),

associated with the efficacy of resveratrol in AD and PD, substantiating our findings through in vitro validation. Collectively, our research contributes to the understanding of the shared pathological features of AD and PD and highlights resveratrol as a potent therapeutic modality that modulates immune and inflammatory pathways for these neurodegenerative disorders.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://portal.gdc.cancer.gov/. The codes related to bioinformatics analysis are available from the corresponding author on reasonable request.

Ethical considerations

All data in this study are from public databases, so no ethical proof is involved.

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CRediT authorship contribution statement

Jinpu Wu: Writing – original draft. **Ziyue Tian:** Writing – review & editing. **Boxue Wang:** Data curation. **Jian Liu:** Methodology. **Ran Bi:** Software, Investigation. **Naixin Zhan:** Validation. **Daixuan Song:** Visualization. **Chengcheng He:** Methodology. **Weimin Zhao:** Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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