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Research Article

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Investigation of the Mechanism of Action of Methyl Hydroxybenzoate in Inducing Alzheimer's Disease Based on Network Toxicology and Molecular Docking

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Abstract

Methylparaben (MeP) is widely used as a preservative; long-term exposure to it has been linked to the onset of neurodegenerative diseases such as Alzheimer's disease (AD). However, the molecular mechanism by which MeP induces AD has not yet been reported. Therefore, this study investigates the potential target molecules and molecular mechanisms underlying MeP-induced AD using computational toxicology and molecular docking methods.Methods: Potential MeP targets were identified using the SwissTargetPrediction, GeneCards, ChEMBL, and STITCH databases; AD-related targets were collected from databases such as OMIM, TTD, and GeneCards to identify the intersection of MeP and AD targets.Using the STRING platform, we constructed a protein-protein interaction (PPI) network from the identified candidate targets; we then used Cytoscape 3.10.3 to visualize the network and perform topological analysis to identify core components and targets;R software version 4.5.0 was used to analyze the intersection of MeP and AD targets for Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses; Autodock 4.2.6 was used to perform molecular docking between MeP and key target proteins, and the results were visualized using PyMOL.Results: A total of 84 overlapping targets between MeP and AD were identified, among which EGFR, ESR1, MMP9, PTGS2, and TP53 were identified as core targets. Molecular docking validated that MeP can stably bind to these core targets.Pathway enrichment results indicate that MeP may influence the onset and progression of AD by modulating neuroactive ligand signaling pathways, neuroactive ligand-receptor signaling pathways, and calcium signaling pathways. This study provides a theoretical basis for the molecular mechanisms by which MeP induces AD and lays the groundwork for the development of preventive and therapeutic strategies for MeP.

Keywords Network toxicology, Molecular docking, Methylparaben (MeP), Alzheimer's disease (AD), Mechanism of action

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1. Introduction

Methylparaben (MeP), one of the most representative members of the parabens class of preservatives, has been widely used as a preservative in foods, pharmaceuticals, cosmetics, and personal care products since the 1920s due to its broad antimicrobial spectrum, chemical stability, and low cost[1, 2] .With the widespread use of MeP, it has been commonly detected in environmental media such as surface water, soil, sediments, and indoor air[3, 4] . The highest concentration of MeP detected in Chinese rivers reached 1,062 ng/L[5] , while the highest concentration in European rivers reached 400 ng/L[6] . In Canadian soil samples, MeP concentrations reached 127 ng/g[7] , and in indoor dust, levels were as high as 1,920 ng/g[8] . Humans are exposed to parabens through multiple routes, including skin contact, dietary intake, and inhalation[9, 10] .In recent years, as these compounds have been widely detected in environmental media and human samples—with detection levels showing an upward trend year by year—paraben esters and their metabolites have been detected in the blood or urine of adults, children, and pregnant women in multiple countries, leading to widespread concern regarding the impact of paraben esters on human health[11, 12] .

Studies have shown that long-term exposure to methylparaben can lead to adverse effects such as endocrine disruption, metabolic disorders, neurotoxicity, reproductive toxicity, and even breast cancer[13–15] .MeP possesses estrogenic activity and affects the endocrine system; long-term exposure can lower thyroid hormone levels and disrupt gene expression in the hypothalamic-pituitary-thyroid axis[16-18] . Animal studies have demonstrated that MeP exposure is closely associated with endoplasmic reticulum stress, inflammatory responses, and glucose-lipid balance, leading to hyperglycemia, hyperlipidemia, and damage to visceral organs[19] .A growing body of evidence indicates that MeP increases oxidative stress in the brain, causing damage to neurons and promoting the development of neurodegenerative diseases[20] . Exposure to MeP leads to damage to neurotransmitter systems, including dopamine receptors and the serotonin system, and even interferes with γ-aminobutyric acid (GABA) activity, affecting behavior and mood[21-23] .MeP binds to the glucocorticoid receptor (GR) and triggers its transactivation, acting on the neuroendocrine system and leading to reduced motor activity in zebrafish larvae[24] ; chronic exposure to MeP results in reduced light sensitivity and memory impairments in adult zebrafish[25] .Recent evidence indicates that MeP accumulates specifically in the liver and adipose tissue of patients with Alzheimer's disease (AD), suggesting its potential involvement in neurodegenerative pathology[26] . As the global incidence of Alzheimer's disease continues to rise and neurodevelopmental disorders become more prevalent, the role of environmental pollutants as "hidden drivers" is receiving increasing attention, although their specific mechanisms of action remain unclear.

Network toxicology integrates bioinformatics, genomics, proteomics, and metabolomics. It utilizes various databases to construct relationship networks among compounds, targets, and pathways, analyzes protein-protein interactions, and predicts the molecular mechanisms by which toxins may lead to disease development. It is particularly well-suited for addressing complex interactions among multiple components, diseases, and targets[27, 28] .Molecular docking can simulate the

interactions between a target compound and biomolecules, predict their affinity and potential biological activity, and assess a compound's toxicity based on the nature and strength of its interactions with targets. The lower the ligand-receptor binding energy, the more stable the ligand-receptor binding conformation, and the greater the likelihood of interaction[29] .This study aims to preliminarily analyze the molecular basis of AD induced by MeP through computational toxicology and molecular docking methods, and to identify potential intervention points with the goal of mitigating its adverse effects.

2. Methods

2.1 Identification of Potential MeP Targets

We searched the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) for "Methylparaben" to identify the standard structural formula of MeP, SMILES notation, and other relevant information, and then entered the MeP compound structure into ProTox (https://tox.charite.de/) for toxicity prediction analysis;We then searched the ChEMBL database (https://www.ebi.ac.uk/chembl/) using the keyword "Methylparaben," restricting the species to "Homo sapiens," to identify potential target genes for MeP; we also entered the MeP compound structure into the STITCH database (http://stitch.embl.de/)and the SwissTargetPrediction database (http://www.swisstargetprediction.ch/), selecting target genes with a predicted likelihood greater than 0 for target prediction. Finally, the target genes predicted by the above three databases were integrated and deduplicated using R version 4.5.0 by creating a Venn diagram.

2.2 Identification of AD-Related Gene Targets

First, based on the GeneCards database (https://www.genecards.org/), the OMIM database (https://www.omim.org/), and the TTD database (https://db.idrblab.net/ttd/), potential targets related to Alzheimer's disease (AD) were screened using "Alzheimer disease" as the search keyword.To ensure a high association between the selected genes and the pathological progression of AD, the "score" threshold for genes in the GeneCards database was set to the median level, retaining only genes with scores above this median threshold.Subsequently, a Venn diagram was generated using R version 4.5.0 for visual analysis. The gene datasets obtained from the three databases were integrated and deduplicated, ultimately identifying the set of genes common to both the MeP and AD target lists; these genes represent the potential targets through which MeP regulates AD.

2.3 PPI Network Construction and Core Target Screening

First, the set of genes common to MeP and AD was imported into Cytoscape 3.10.3 to construct a compound-gene-disease regulatory network, with the aim of elucidating the molecular mechanism by which MeP mediates the regulation of AD through these genes.Subsequently, this set of overlapping genes was submitted to the STRING database (https://cn.string-db.org/) to construct a raw protein-protein interaction (PPI) network. The species was set to Homo sapiens (TaxID: 9606),set the minimum interaction confidence threshold to high confidence (>0.4), and hid isolated nodes to remove redundant genes without interactions. We then exported the network file and attribute file in TSV format.After importing the above files into Cytoscape 3.10.3, we performed visual analysis of molecular interactions by calculating node topological parameters (degree, betweenness centrality, closeness centrality) to quantitatively characterize the topological properties of network nodes and edges, ultimately generating a high-quality PPI network diagram. Core targets were selected based on the following topological parameter thresholds: ① betweenness centrality > median;② Clustercentrality > median; ③

Degree > upper quartile (Q3). Nodes meeting all of the above conditions are defined as core targets.

2.4 Gene Ontology (GO) Function and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

Using R version 4.5.0, we analyzed the common targets of MeP and AD to perform Gene Ontology (GO) enrichment analyses for biological processes (BP),molecular function (MF), and cell component (CC) enrichment analyses, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The results were then sorted from highest to lowest by the number of enriched genes to identify the primary gene functional enrichment processes and signaling pathways induced by MeP in AD, thereby predicting its potential mechanisms of action.

2.4 Molecular Docking

Three-dimensional crystal structure files (in PDB format) of core target proteins were retrieved and downloaded from the RCSB Protein Data Bank (RCSB PDB,https://www.rcsb.org/) to retrieve and download the 3D crystal structure files (PDB format) of core target proteins. We used AutoDock 4.2.6 software to perform molecular docking experiments between MeP and the core target proteins, aiming to predict their binding affinities and obtain the lowest binding energy values and key binding site information for each target protein.Subsequently, PyMOL software was used to perform a visual analysis of the docking results, systematically analyzing the characteristics of intermolecular interactions (including the specific formation of hydrogen bonds, van der Waals forces, ionic bonds, and other forces) to clarify the specific binding patterns between MeP and the active pockets on the surfaces of the core target proteins.

3. Results

3.1 MeP Toxicity Prediction and Identification of Predicted Targets

A search for “Methylparaben” on the PubChem website was conducted to identify the compound structure of MeP and related information, such as SMILES notation. The MeP compound structure was then entered into ProTox for toxicity prediction analysis. The lower the toxicity level, the higher the toxicity. The results showed that MeP has a toxicity level of 4, indicating moderate toxicity, and predicted toxicity to the liver, brain, and kidneys, with the strongest toxicity observed in the brain (see Figure 1).A search for “Methylparaben” was conducted in the ChEMBL database, and the MeP compound structure was entered into the STITCH and SwissTargetPrediction databases for retrieval. Potential target molecules were screened and extracted, and the target data obtained from the database searches were

merged and deduplicated (i.e., a union was taken to integrate all potentially associated targets). Ultimately, a core set of 134 potential target molecules was identified, as shown in Figure 2.

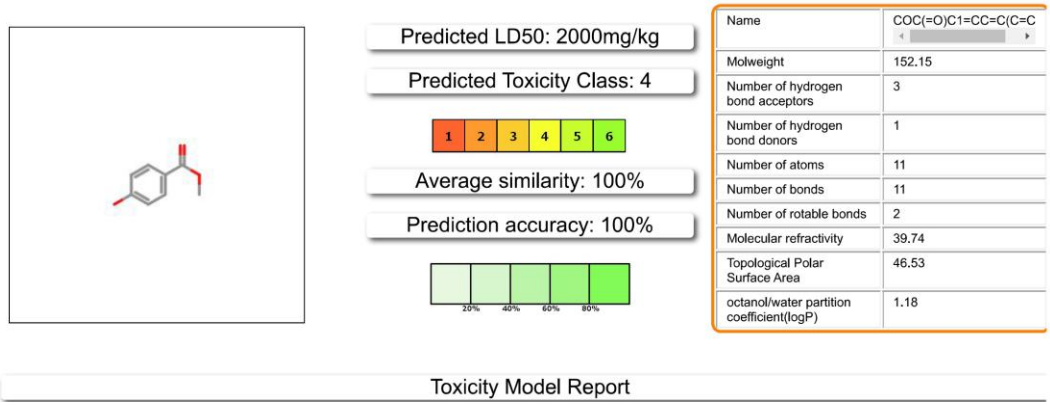


Figure 1. Mep Toxicity Prediction

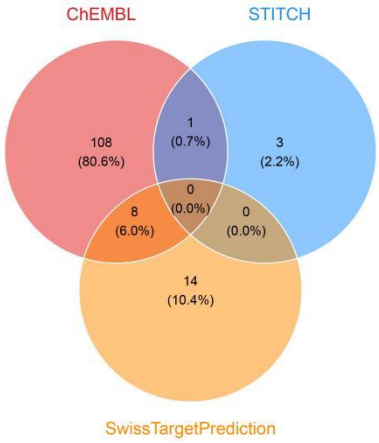


Figure 2 Potential MeP Targets

3.2 Screening of AD Targets and Identification of Targets Overlapping with MeP

Using “Alzheimer’s disease” as the search keyword, a systematic search was conducted across the GeneCards, TTD, and OMIM databases to screen for potential targets associated with Alzheimer’s disease (AD).The results from the multi-database search were merged and deduplicated (union operation), ultimately yielding 3,329 potential AD-related gene targets. The standard gene names corresponding to each target were also extracted; see Figure 3 for details.To identify common targets between MeP and AD, a Venn diagram was generated using R version 4.5.0 to analyze the intersection between MeP’s potential targets and the aforementioned AD-related gene targets. The results showed a total of 84 overlapping targets; see Figure 4 for details.

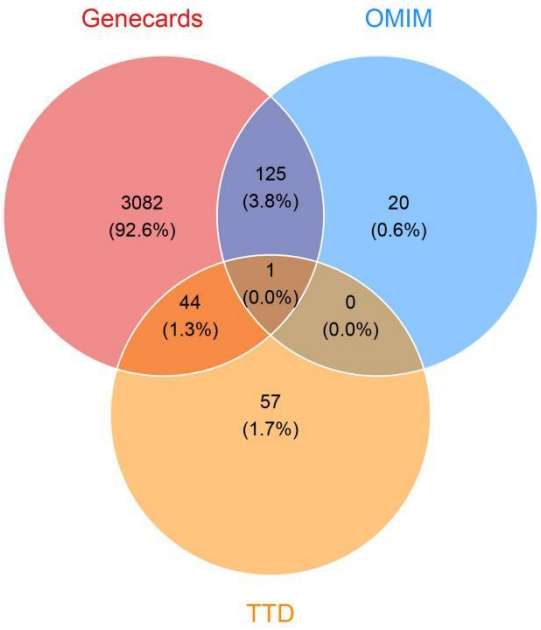


Figure 3: AD Gene Targets

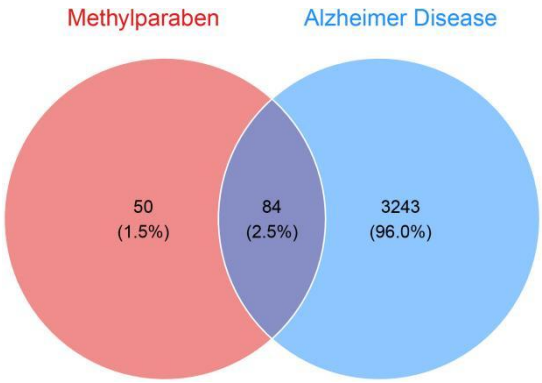


Figure 4: Overlapping Targets of MeP and AD

3.3 Construction of the MeP–AD PPI Network

The 84 targets from MeP and AD were imported into the STRING platform for analysis to construct a PPI network diagram of MeP and AD targets (Figure 5(a)). A visual analysis of this protein-protein interaction (PPI) network was then performed based on degree values; the results are shown in Figure 5(b).Here, the "Degree" value characterizes the connectivity of nodes in the network; the higher the node's degree, the more critical its role in the network's regulatory pathways.The PPI network analysis results show that the TP53, EGFR, ESR1, MMP9, PTGS2, MAPK3, and DRDA genes exhibit denser connectivity within the network; they occupy central regulatory positions in the PPI network and serve as key node genes within it. The resulting network file was imported into Cytoscape for the visual representation of compounds, diseases, and targets (Figure 5(c)).

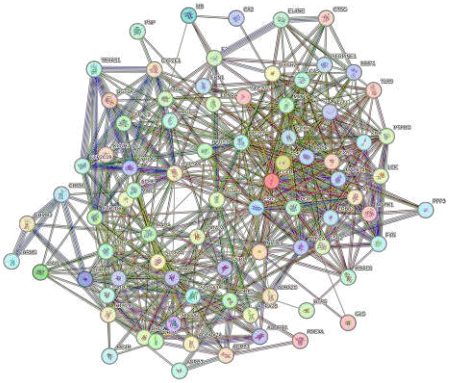


Figure 5(a) Protein-protein interaction network diagram

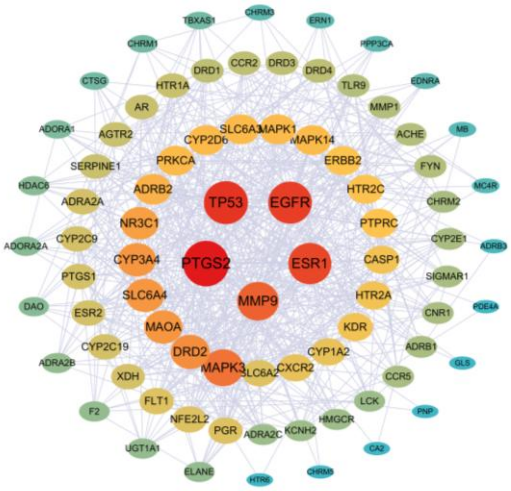


Figure 5(b) PPI network diagram

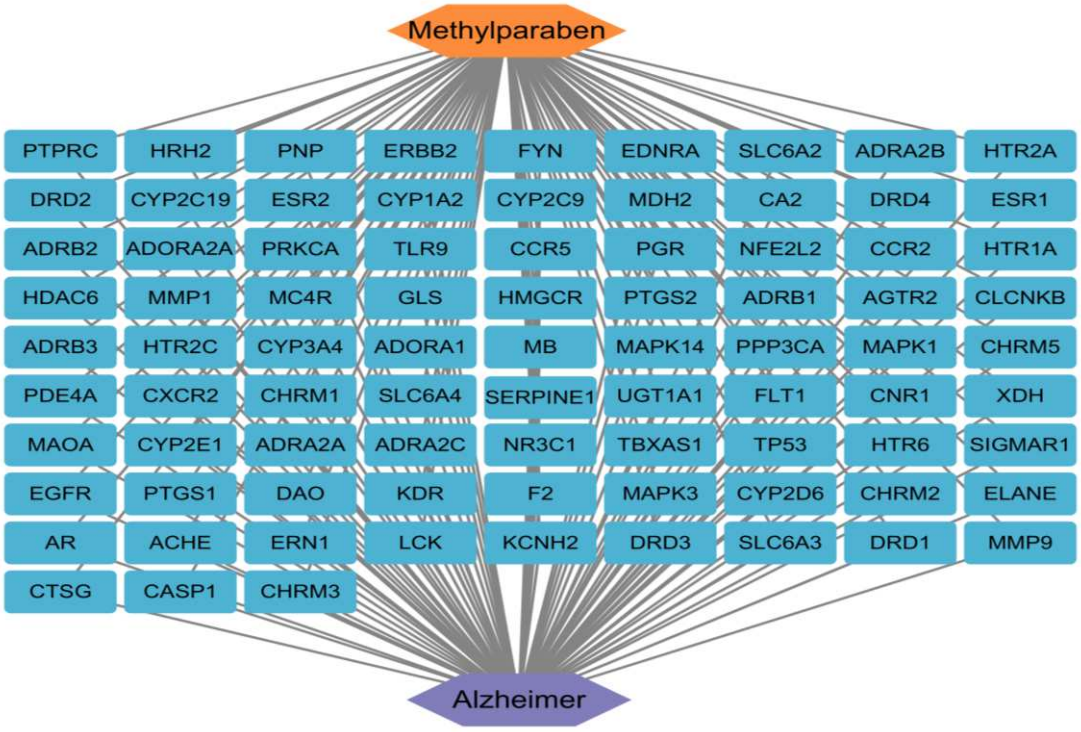


Figure 5(c) MeP-AD-target diagram

3.4 GO Functional and KEGG Enrichment Analysis

To systematically analyze the mechanism by which MeP induces AD, we used R version 4.5.0 to analyze the 84 potential intersections between MeP and AD,The results showed that across the three dimensions of GO functional annotation: Biological Process (BP) yielded a total of 2,905 enriched entries, Cellular Component (CC) yielded 211 enriched entries, and Molecular Function (MF) yielded 345 enriched entries.After sorting the enriched entries in these three dimensions according to relevant statistical metrics (such as enrichment

fold and P-value), the top 10 entries from each dimension were selected for display, ultimately yielding 30 core GO annotation entries.Among these, BP is primarily associated with reactive oxygen species metabolism, response to lipopolysaccharides, regulation of reactive oxygen species metabolism, cellular response to chemical stress, exogenous apoptosis signaling pathways, negative regulation of apoptosis signaling pathways, response to reactive oxygen species, and regulation of apoptosis signaling pathways;CC is primarily associated with membrane rafts, membrane microstructures, membrane regions, vesicle lumens, secretory granule lumens, cytoplasmic vesicle lumens, protein kinase complexes, and full-length cell cycle protein-dependent kinase complexes;MF's core associated functions include cytokine receptor binding, cytokine activity, receptor-ligand interaction, signal receptor activator activity, and DNA-binding transcription factor binding, as shown in Figure 6(a). A KEGG pathway enrichment analysis was performed on the overlapping gene targets between MeP and AD,the results revealed a total of 228 enriched KEGG pathway entries. The top 20 AD-related pathways were identified, involving neuroactive ligand signaling, neuroactive ligand-receptor interactions, calcium signaling pathways, serotonergic synapses, chemocarcinogenesis—receptor activation, hormone signaling, cAMP signaling, and other pathways, as shown in Figure 6(b).Analysis of the above enrichment results suggests that MeP may induce the onset of Alzheimer's disease (AD) through multiple processes and pathways. Among these, neuroactive ligand signaling, neuroactive ligand-receptor signaling, and calcium signaling are core pathways. Based on this, it is hypothesized that these pathways are most likely involved in the process by which MeP induces AD.

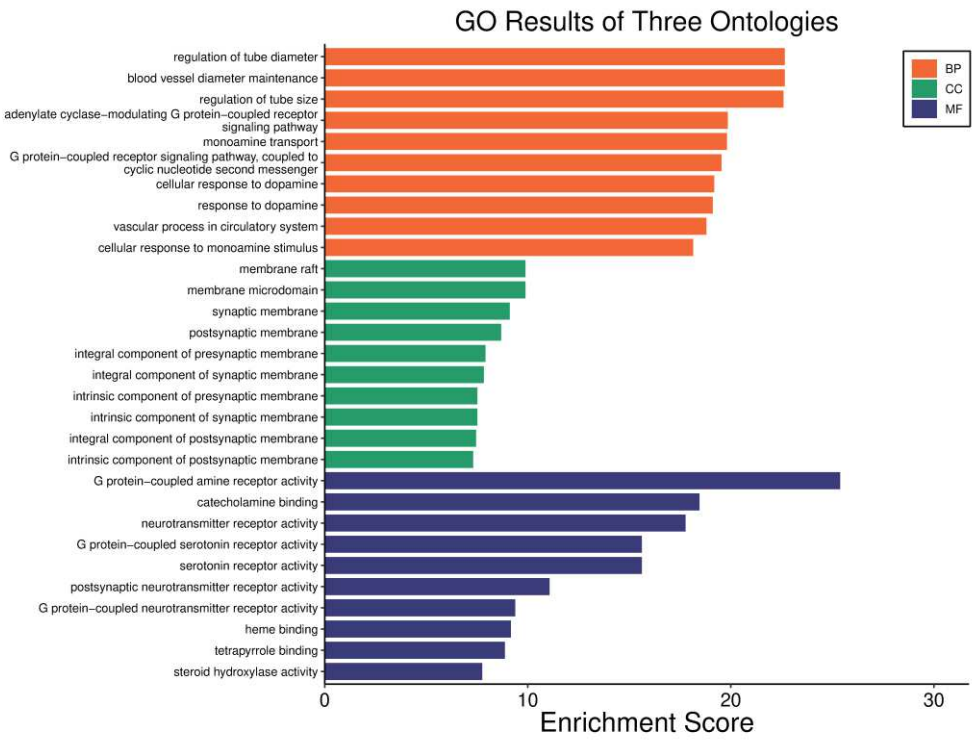


Figure 6(a) GO enrichment analysis of relevant targets

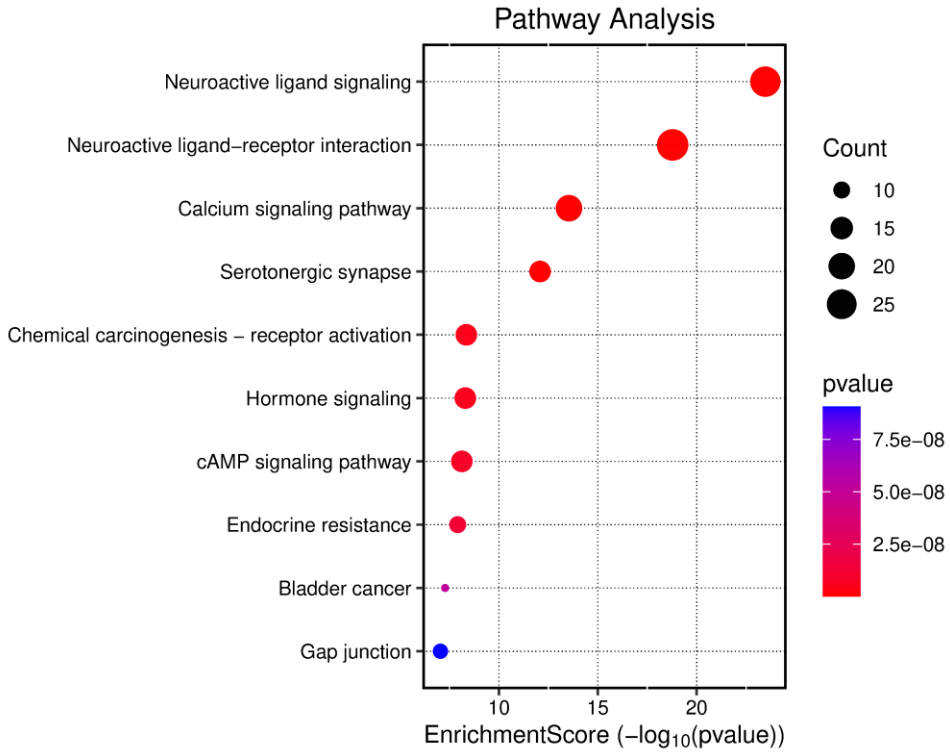


Figure 6(b) KEGG pathway enrichment analysis

3.5 Molecular Docking

To validate the interaction characteristics between MeP and core target proteins, this study conducted molecular docking experiments, using binding energy as a quantitative indicator of docking efficacy—the higher the absolute value of binding energy, the stronger the binding affinity between the ligand (MeP) and the target protein.The experiments focused on five core target proteins (EGFR, ESR1, MMP9, PTGS2, and TP53) to elucidate the interaction characteristics between MeP and these targets. Generally, when the lowest binding energy is below −5 kJ/mol, the binding activity between the ligand and the target protein is considered good.The results show that the minimum binding energies of MeP with the aforementioned five target proteins all meet this criterion, specifically: EGFR (-5.9 kJ/mol), ESR1 (-5.7 kJ/mol), MMP9 (-6.5 kJ/mol), PTGS2 (-6.2 kJ/mol), and TP53 (-6.2 kJ/mol).The above docking results confirm that MeP exhibits strong binding affinity with the five core target proteins. The binding energies of all proteins with MeP are less than 0, indicating that MeP can spontaneously bind to the core targets. Furthermore, hydrogen bond interactions are present when MeP binds to these target proteins (see Figure 7), suggesting that they play a crucial role in the molecular mechanism by which MeP induces AD.

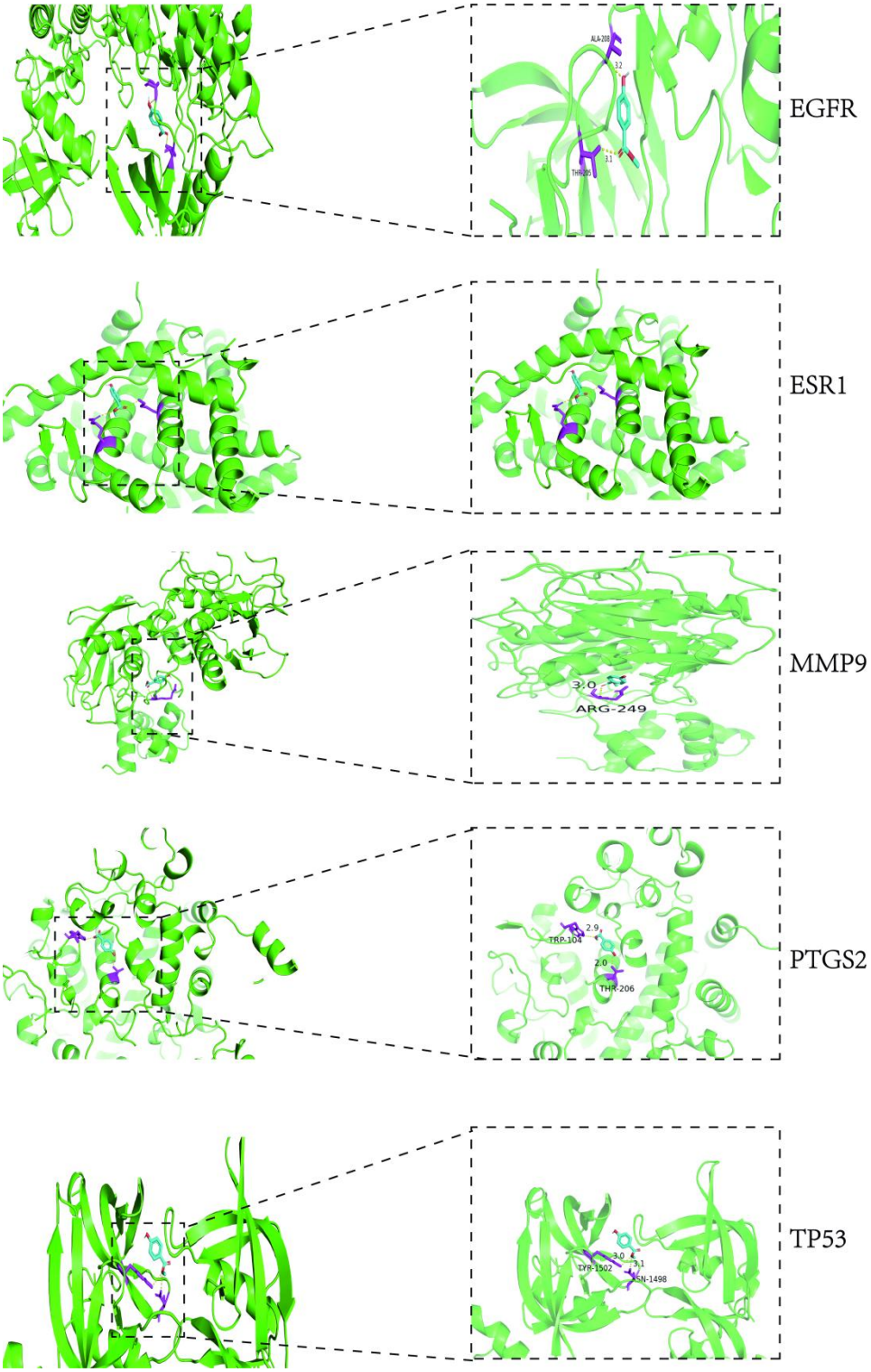


Figure 7 Molecular docking diagrams of MeP with EGFR, ESR1, MMP9, PTGS2, and TP53

4. Discussion

This study employed a network toxicology approach for target screening, identifying a total of 134 MeP-related targets and 3,329 AD-related targets, with 84 targets in their intersection.Based on these results, further screening via PPI network topological analysis identified TP53, EGFR, ESR1, MMP9, PTGS2, and others as core targets, which play important roles in cellular damage, apoptosis, and inflammatory responses.TP53 is a multifunctional transcription factor that, after

binding to its target DNA sequences, participates in DNA repair, senescence, cell cycle control, autophagy, and apoptosis; it induces growth arrest or apoptosis depending on physiological conditions and cell type, and negatively regulates cell division by controlling the expression of a set of genes required for these processes[30] .TP53 has been reported to colocalize with cytoplasmic microtubules in a Ca^{2+}-dependent manner, and Ca^{2+} exerts a potentially inhibitory effect on TP53 activity. Another possible mechanism of Ca^{2+}-dependent TP53 regulation is calcium-activated protease-induced TP53 cleavage; calcium-activated proteases are involved in various cellular processes, including proliferation, apoptosis, and migration[31, 32] .EGFR is a specific transmembrane glycoprotein that, upon binding to its ligand, activates cytoplasmic tyrosine kinase activity and triggers the signaling of multiple downstream pathways, thereby stimulating the differentiation and proliferation of astrocytes. EGFR can also stimulate astrocyte activation by phosphorylating STAT3, leading to neuronal damage[33] ;EGFR activates protein kinase C, leading to its hydrolysis into inositol triphosphate (IP3). IP3 mobilizes intracellular Ca^{2+}, establishing a link between receptor phosphorylation and Ca^{2+}, thereby affecting calcium signaling pathways and impairing cognitive function[34] .ESR1 is the estrogen receptor, and the relationship between estrogen levels and AD and cognitive performance remains controversial. A longitudinal study on the association between baseline serum estrogen levels and dementia risk found that high estrogen levels in postmenopausal women were associated with a higher risk of AD; however, female AD patients with higher estrogen levels exhibited smaller hippocampal volume and poorer memory function[35] ;Thal and colleagues conducted a study on the correlation between estrogen levels and cognition in 120 postmenopausal female AD patients receiving estrogen therapy. They found that while hormone levels rose significantly after estrogen therapy, there were no corresponding changes in neuropsychological test scores.During nervous system development, GABA influences neuronal migration, proliferation, and differentiation. Traditional research suggests that estrogen receptors are primarily expressed in GABAergic interneurons[37] .Matrix metalloproteinase 9 (MMP9), a core member of the matrix metalloproteinase (MMP) family, is primarily localized in neurons, glial cells, and vascular endothelial cells; furthermore, as one of the key downstream effector molecules in inflammatory responses, its upregulation is significantly positively correlated with the initiation and progression of inflammation.Upon activation, MMP9 can cross the blood-brain barrier to cause damage to brain tissue cells, exacerbate the severity of neurological deficits, and increase the risk of cognitive impairment[38] . PTGS2 (COX-2) is a key enzyme catalyzing the rate-limiting step in the synthesis of various inflammatory prostaglandins and is widely distributed in the body[39] .Studies have shown that PTGS2 can reflect the severity of brain injury; compared with healthy individuals, PTGS2 expression is upregulated in the serum and cerebrospinal fluid of patients with Alzheimer’s disease (AD) and mild cognitive impairment (MCI)[40] . Research by Pu Wang[41] and others has confirmed that COX-2 is activated during the development and progression of AD and may be involved in the pathogenesis of the disease.COX-2 promotes the

diffusion and deposition of Aβ in cerebellar β-amyloid plaques and leads to excessive phosphorylation of tau protein; therefore, COX-2 may be a potential therapeutic target for [AD] . In summary, the pathological mechanisms of AD are complex, and identifying the corresponding key targets is of great significance for exploring how to prevent and treat AD.

This study also found that the common pathways involved in MeP-induced AD primarily include neuroactive ligand signaling pathways, neuroactive ligand-receptor signaling pathways, and calcium signaling pathways; these pathways may represent the primary signaling routes underlying MeP-induced AD. Downregulation of dopamine receptors significantly accelerates protein translation within neurons, promotes Aβ accumulation, and affects behavioral and cognitive functions[43] .Gamma-aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT) are important neurotransmitters in the central nervous system. They transmit inhibitory signals by binding to and activating their corresponding G protein-coupled receptors and downstream signaling pathways; low levels of GABA and 5-HT exacerbate cognitive impairment[44, 45] .Studies have demonstrated that long-term exposure to MeP causes damage to various neurotransmitter systems—including dopamine receptors, the serotonin system, and γ-aminobutyric acid (GABA)—thereby affecting neuroactive ligand signaling pathways and leading to cognitive decline.Calcium signaling is one of the key forms of cellular signal transduction; its core function is to mediate transmembrane communication between the intracellular and extracellular environments. It often acts as a second messenger in living organisms and is involved in many cellular processes essential to neuronal survival. A growing body of research evidence indicates that dysfunction of calcium signaling pathways plays a critical role in the pathogenesis of Alzheimer's disease (AD).Studies have shown that the excessive Ca^{2+} release from the endoplasmic reticulum (ER) and elevated cytoplasmic Ca^{2+} concentrations observed during AD may be due to the dysregulation of intracellular calcium signaling in Alzheimer's disease involving antioxidant and redox signaling[.Ca^{2+} channel activity plays a crucial role in neuronal survival and death through Ca^{2+}-regulated nuclear transcription signaling; modulating the Ca^{2+}-dependent CaMKII/CREB signaling pathway in juvenile AD mice can reverse synaptic defects[47] , and animal studies have demonstrated that influencing calcium signaling pathways can activate neuroinflammation, leading to cognitive impairment[48] .

5. Conclusion

This study explored the mechanism by which MeP exerts its effects on AD through network toxicology combined with molecular docking analysis. The results indicate that MeP-induced AD follows a complex regulatory pattern involving multiple components, targets, and pathways; its potential mechanism of action may involve TP53,EGFR, ESR1, MMP9, and PTGS2, to regulate key pathways—including neuroactive ligand signaling, neuroactive ligand-receptor signaling, and calcium signaling—ultimately leading to cognitive decline. These findings provide evidence for further elucidation of the molecular mechanisms underlying MeP-induced AD, laying the groundwork for future in-depth research.

Supplementary Information

Author contribution Morong Guo and Jun Zhu designed

the study. Bin Wen and Yuyu Shaoanalyzed the data and wrote the first draft of the manuscript. Lan Yang collected the data. Morong Guo revised the manuscript. All authors readand approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability All data analyzed in this study have been presented in this article and the supplementary information.

Declarations

Ethical approval Not applicable.

Conflict of interest The authors declare no competing interests.

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