Original
ISSN 2413-0516

Molecular Mechanisms of Melatonin in Alzheimer's Disease: Insights from Network Pharmacology and Molecular Docking

Shima Mohammadi[1], Simin Mahakizadeh[2], Mahnaz Poorhassan[3], Gholamreza Hassanzadeh[4,5,6*]

[1]Department of Neuroscience, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.
[2]Department of Anatomy, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran.
[3]Department of Anatomical Sciences and Cognitive Neuroscience, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
[4]Department of Neuroscience and Addiction Studies, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[5]Department of Digital Health, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[6]Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
*Correspondence to: Gholamreza Hassanzadeh (E-mail: hassanzadeh@tums.ac.ir)
(Submitted: 20 November 2024 – Revised version received: 19 December 2024 – Accepted: 10 January 2025 – Published online: 26 February 2025)

Abstract

Objective: This study explores melatonin's potential therapeutic effects on Alzheimer's disease (AD) using computational tools, focusing on its impact on key pathological features of AD.

Methods: We assessed ML's pharmacological properties, toxicity, and biological targets, identifying overlaps with AD-related genes. A pharmacological-target-pathology network was developed utilizing the software Cytoscape, and an analysis of protein-protein interactions (PPI) was conducted employing the STRING database. Gene Ontology (GO) and KEGG pathway enrichment analyses were conducted, followed by protein-ligand docking simulations to validate ML's interactions with key targets.

Results: ML exhibits favorable ADME properties, good solubility, and the ability to cross the blood-brain barrier, with a generally safe toxicity profile; however, caution is advised regarding neurotoxicity and respiratory toxicity. Our analysis identified 15,564 AD-related genes and 101 ML targets, with 95 shared genes. Key genes in the PPI network include EGFR, PTGS2, ERBB2, and others. GO analysis highlighted processes related to nitrogen compounds, cell proliferation, and membrane functions, particularly in serotonin receptor signaling. Molecular docking revealed ERBB2 as the strongest ML target, suggesting its potential in AD therapy.

Conclusions: This study concludes that ML may offer a promising therapeutic approach for AD by targeting multiple pathways and key proteins, such as ERBB2, and modulating biological processes related to neuronal signaling.

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

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative and metabolic disorder characterized primarily by the degeneration of neuronal cells, particularly in the cerebral cortex and hippocampus.[1] This neuronal loss leads to a gradual deterioration of memory, cognitive functions, and emotional regulation, manifesting as depression, anxiety,[2] and ultimately progressing to dementia.[3] As a multifactorial condition, AD is initiated by molecular mechanisms involving the accumulation of amyloid-beta (Aβ) plaques, hyperphosphorylated tau proteins, and the formation of neurofibrillary tangles, which are considered pivotal in the early stages of the disease.[4,5] A growing body of research has recently focused on the role of neurotransmitter dysfunction and receptor abnormalities in the pathogenesis of AD.[6] Specifically, disruptions in both cholinergic neurotransmitters, such as gamma-aminobutyric acid (GABA) and acetylcholine (ACh), and monoaminergic neurotransmitters, including serotonin dopamine (DA), (5-HT), and melatonin (ML), have been implicated in the AD progression.[4] These dysregulations are closely associated with the pathological features of AD, as they trigger the activation of various molecular pathways that contribute to neurodegeneration.

Furthermore, the central nervous system (CNS) in individuals with AD demonstrates significantly elevated levels of reactive oxygen and nitrogen species.[7] This oxidative stress is exacerbated by factors such as increased metabolic activity, imbalances in transition metal concentrations, and elevated levels of unsaturated fatty acids.[8] Another defining characteristic of neurodegenerative disorders is the dysregulation of inflammatory processes.[9,10] This is marked by the uncontrolled activation of microglia, which subsequently release proinflammatory cytokines. These inflammatory mediators contribute to the cascade of events leading to neuronal cell death, further exacerbating the neurodegenerative process.[11]

The therapeutic potential of cholinesterase inhibitors, such as memantine, donepezil, rivastigmine, and galantamine, is well established in the management of AD.[12] These agents inhibit cholinesterase activity, preventing the breakdown of acetylcholine and enhancing its activity, which improves long-term memory and learning. However, while these drugs alleviate symptoms, they do not reverse the underlying pathology of AD, as the acetylcholine pathway primarily addresses symptom management rather than disease modification.[4,13] Therefore, there is a growing interest in exploring alternative therapeutic strategies that target the underlying pathological mechanisms of AD.

One promising candidate in AD research is ML (N-acetyl-5-methoxytryptamine), a serotonin-derived hormone synthesized in the pineal gland.[14] ML plays a critical role in regulating circadian rhythms, reducing oxidative stress, and enhancing immune function.[15,16] Its decline in AD patients has been associated with sleep disturbances and disruptions in circadian rhythms, underscoring its potential as a therapeutic target.[17] Although research on ML's effects on neurotrmokhansmitters and its efficacy in AD treatment remains limited compared to cholinergic drugs, its low toxicity and ability to modulate cholinergic and glutamatergic systems position it as a promising alternative to cholinesterase inhibitors.[18] Notably, ML exhibits

neuroprotective properties by inhibiting amyloid precursor protein synthesis and preventing the formation of Aβ plaques, further supporting its therapeutic potential in AD.[19] However, the molecular pathways through which ML exerts its effects in AD treatment are not yet fully understood. To address this gap, the integration of bioinformatics offers a transformative approach.

Network pharmacology enable the analysis of complex biological networks and large-scale data to identify drug targets, elucidate mechanisms of action, and map signaling pathways.[20,21] While network pharmacology is increasingly applied to study pharmacological effects, drug efficacy, and disease mechanisms, it remains a developing field that requires further standardization and refinement of evaluation metrics and methodologies.[22] Its potential to advance the understanding of disease pathways and develop targeted therapies underscores its critical role in modern biomedical research, particularly in elucidating the mechanisms of promising candidates.[23]

By leveraging ML as a potential therapeutic agent for AD, our work aims to provide novel insights and theoretical foundations for identifying active compounds in ML and elucidating its mechanisms in AD prevention and treatment. Through network pharmacology, protein-protein interaction (PPI) analysis, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and molecular docking, we seek to uncover key molecular targets and signaling pathways, advancing the understanding of ML's therapeutic potential in AD.

Methods and Materials

ADME and Toxicity

In this research, we examined the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of ML using data sourced from SwissADME (http://www.swissadme.ch/index.php). This web-based platform offers extensive insights into the physicochemical and pharmacokinetic profiles of chemical compounds.[24] Our analysis concentrated on critical parameters such as physicochemical attributes, lipophilicity, aqueous solubility, pharmacokinetics, and druglikeness, with the aim of understanding their impact on ML's therapeutic potential. Furthermore, ProTox-II (https://tox-new.charite.de/) was employed to assess ML's toxicity profile.[25]

Screening of Target Genes

The biological targets of ML were obtained from swisstargetprediction (http://www.swisstargetprediction.ch/) using SMILE. The targets of AD were obtained from OMIM (https://www.omim.org/). Similarly, we used the UniProt ID mapping tools to convert the target protein IDs into their corresponding gene symbols. Next, the overlapping gene targets of ML and AD were identified using InteractiVenn (Union by list) (https://www.interactivenn.net/). These shared targets were then analyzed further to identify potential candidates for AD treatment.

Drug-Target-Disease Network

In our research, we employed Cytoscape 3.10.2 to create a drug-target-disease network that illustrates the interactions between the targets associated with AD and ML. Cytoscape is a robust open-source software platform designed for visualizing complex biological networks, allowing us to integrate various data types and gain insights into the relationships between drugs, their targets, and related diseases.[26] By mapping the interactions between ML's targets and those implicated in AD, we were able to identify potential therapeutic pathways and highlight how ML may influence the progression or treatment of AD.

Protein-Protein Interaction

In our investigation, we performed a PPI analysis using STRING version 12 (https://string-db.org/), an online database designed to predict PPI networks.[27] We input the common targets of ML related to AD into the STRING database, which allowed us to generate comprehensive PPI networks along with relevant data. To elucidate the mechanisms through which ML may exert its effects against AD, we constructed a network that integrates components, diseases, targets, and pathways using Cytoscape software (version 3.10.2). In this network, nodes represent components, targets, pathways, and diseases, while edges illustrate the interactions between proteins. To analyze the network's structure, we used the CytoNCA App, which allowed us to calculate degree, betweenness, and closeness centrality. This analysis helped us identify the top ten genes with the highest degree of connectivity within the network.

Enrichment Analysis of the Intersected Genes

To better understand the roles of the ten key intersected targets, we performed GO analysis, covering biological processes (BP), cellular components (CC), and molecular functions (MF). Additionally, KEGG pathway enrichment analysis was carried out using ShinyGO version 0.81.[28] The top 20 enriched terms from each analysis were visualized. For both GO and KEGG analyses, pathways and terms with a false discovery rate (FDR) below 0.05 were considered significantly enriched. This thorough approach enhances our understanding of the biological relevance of these targets in relation to ML's effects on AD.

Molecular Docking

The five key genes identified in our study were selected as primary targets for receptor interactions. Structural information for these receptors was sourced from the RCSB Protein Data Bank (RCSB PDB), including the following: EGFR (PDB ID: 4G5J), PTGS2 (COX-2, PDB ID: 3LN1), ERBB2 (PDB ID: 3PP0), HTR1A (PDB ID: 7E2X), and PARP1 (PDB ID: 7KK3, chain C). Additionally, data from the UniProt database was utilized to complement our analysis. The 2D structures of MEL were obtained from the PubChem database in .sdf format, and these optimized ligands served as the starting points for molecular docking studies. We performed protein-ligand docking calculations using CB-DOCK2 (https://cadd.labshare.cn/cb-dock2/php/blinddock.php#job_list_load).[29] Throughout this process, we calculated affinity scores, with scores of -5 or lower indicating high affinity. After completing the docking search, we visualized the binding sites and the amino acids involved in the ligand-receptor interactions using CB-DOCK2.

Results

ADME and Toxicity

The analysis of MT's (Figure 1A) ADME properties reveals several key characteristics that contribute to its effectiveness

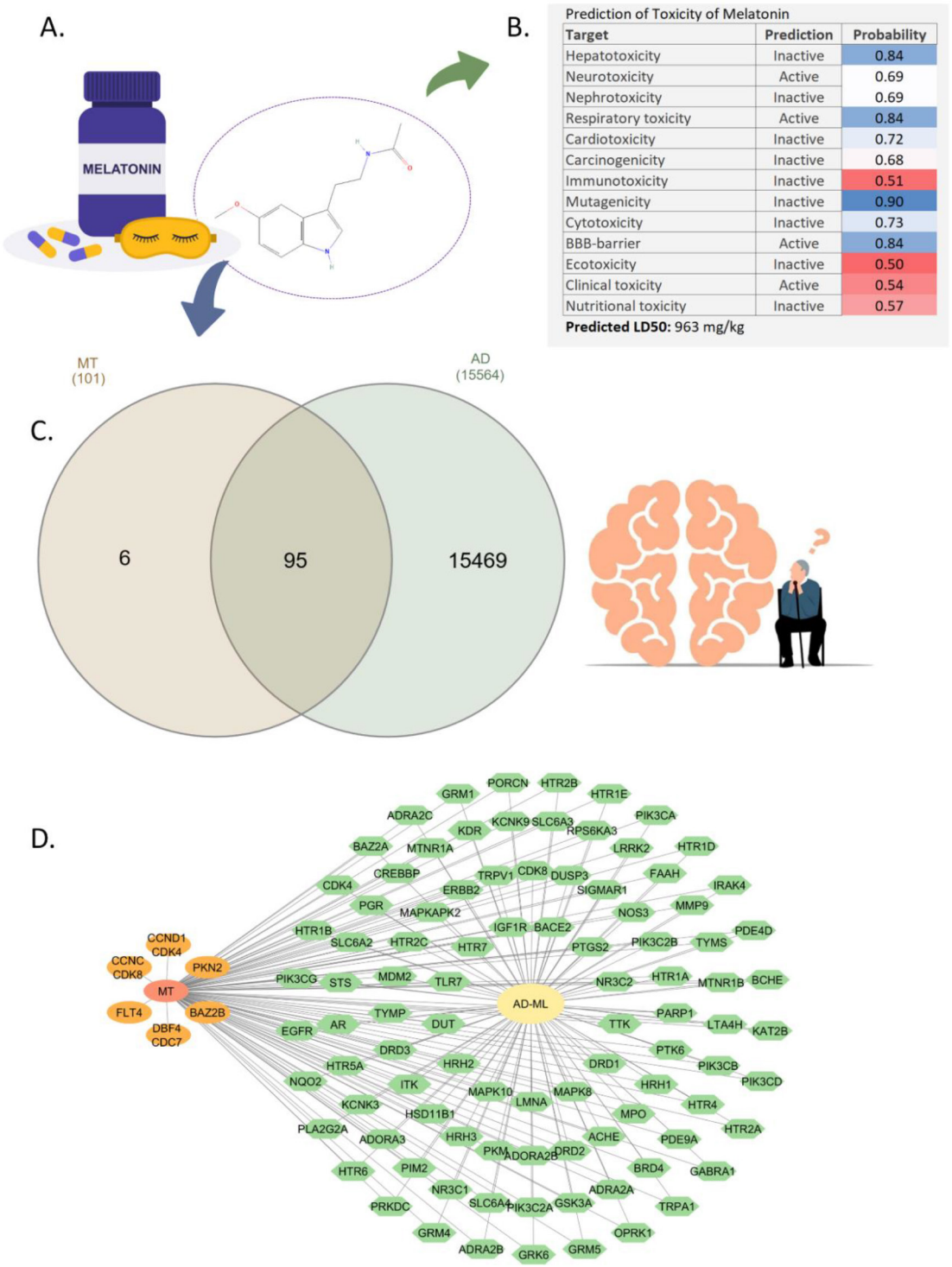


Fig. 1 **Pharmacological characteristics of ML and its target-disease network. A. Chemical structure of MT; B. Toxicity predictions for MT; C. Venn diagram illustrating common targets between MT and AD; D. Analysis of the target-disease network pharmacology associated with MT.**

as a therapeutic agent. The physicochemical properties indicate a well-balanced molecular structure, with a molecular weight of 232.28 g/mol and a moderate Topological Polar Surface Area (TPSA) of 54.12 Å². These features suggest that MT can effectively interact with biological targets while maintaining sufficient solubility and permeability. Additionally, its moderate lipophilicity, as evidenced by a consensus Log Po/w of 1.83, facilitates its absorption in the gastrointestinal tract and its ability to cross cell membranes, which is crucial for its therapeutic effects. Additionally, MT exhibits an advantageous pharmacokinetic profile, including high gastrointestinal absorption and effective permeability across the blood-brain barrier (BBB). The compound is not a substrate for P-glycoprotein, enhancing its effectiveness by reducing the likelihood of being actively transported out of cells. Additionally, MT's classification as an inhibitor of CYP1A2 suggests potential interactions with other drugs, which may influence its metabolic pathways. Overall, these properties underscore MT's

suitability as a therapeutic agent, particularly in the context of AD and related physiological processes (Table 1). The toxicity profile of MT reveals a varied risk across different areas. It is deemed inactive for hepatotoxicity, nephrotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and nutritional toxicity, indicating a lower likelihood of adverse effects in these categories. However, it shows active predictions for neurotoxicity, respiratory toxicity, and clinical toxicity, suggesting potential concerns in these areas. With a predicted LD50 of 963 mg/kg, MT generally appears safe, but caution is warranted regarding its effects on the nervous and respiratory systems (Figure 1B).

Table 1. **ADME of MT**

Physicochemical properties	
Formula	C13H16N2O2
Molecular weight	232.28 g/mol
Num. heavy atoms	17
Num. arom. heavy atoms	9
Fraction Csp3	0.31
Num. rotatable bonds	5
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	67.18
TPSA	54.12 Å^2
Lipophilicity	
Log Po/w (iLOGP)	1.98
Log Po/w (XLOGP3)	1.59
Log Po/w (WLOGP)	1.86
Log Po/w (MLOGP)	0.97
Log Po/w (SILICOS-IT)	2.78
Consensus Log Po/w	1.83
Water Solubility	
Log S (ESOL)	–2.34
Solubility	1.05e+00 mg/ml; 4.53e-03 mol/l
Log S (Ali)	–2.34
Solubility	1.07e+00 mg/ml; 4.60e-03 mol/l
Log S (SILICOS-IT)	–4.62
Solubility	5.62e-03 mg/ml; 2.42e-05 mol/l
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log Kp (skin permeation)	–6.59 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55

Prediction of Common Targets

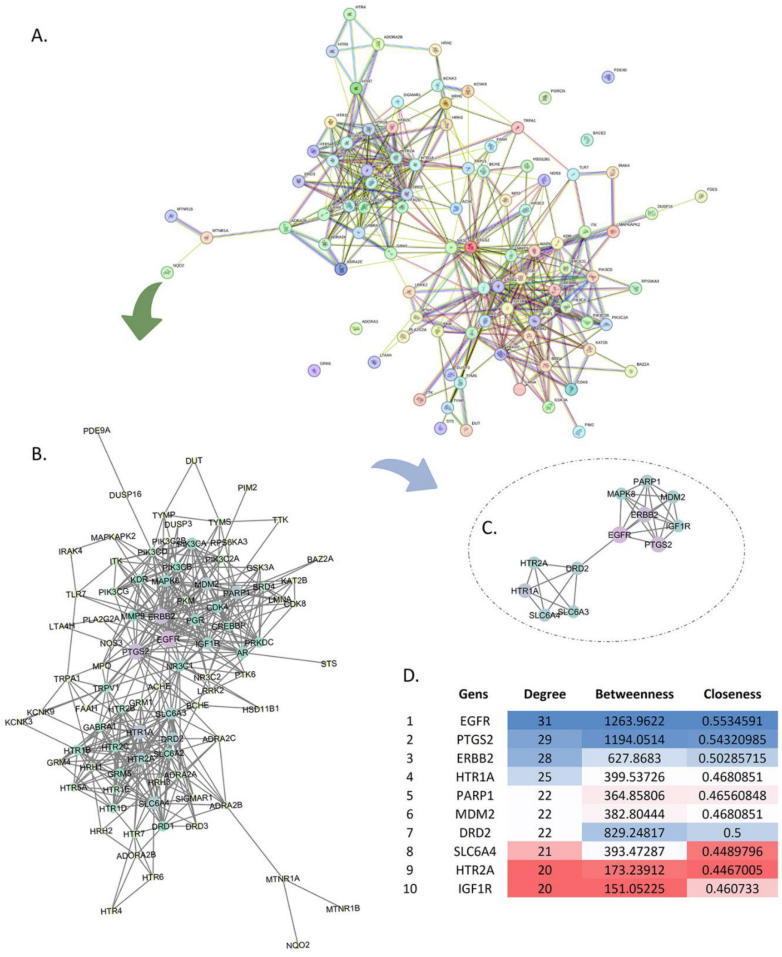
Our analysis identified 15,564 genes associated with AD pathogenesis and 101 targets linked to ML. Using a Venn diagram, we identified 95 overlapping targets between ML and AD, as shown in Figure 1C. To further investigate these shared targets, we conducted a network analysis to visualize the interactions between ML-related targets and AD-associated genes, with the 95 common targets prominently displayed in Figure 1D. This analysis underscores the therapeutic potential of ML in AD and provides a foundation for further investigation into these shared targets.

PPI Network

In the PPI network analysis focusing on ML and AD, we identified several key genes that exhibited a high degree of connectivity within the network using STRING platfrom. Notable among these are EGFR (Epidermal Growth Factor Receptor), PTGS2 (Prostaglandin-Endoperoxide Synthase 2), ERBB2 (Erb-B2 Receptor Tyrosine Kinase 2), HTR1A (5-Hydroxytryptamine Receptor 1A), PARP1 (Poly (ADP-ribose) Polymerase 1), important for DNA repair; MDM2 (Mouse Double Minute 2 Homolog), DRD2 (Dopamine Receptor D2), SLC6A4 (Solute Carrier Family 6 Member 4), HTR2A (5-Hydroxytryptamine Receptor 2A), and IGF1R (Insulin-like Growth Factor 1 Receptor). The high degree of these genes indicates their potential significance in the biological pathways connecting ML's effects to the mechanisms underlying AD, suggesting they may serve as important therapeutic targets for further investigation (Figure 2).

GO and KEGG

Using ShinyGO version 0.81, we conducted an extensive analysis of GO and KEGG pathways to investigate the functional roles of the top ten intersected targets associated with ML and AD. The GO analysis identified 1,000 biological processes (BP), with the top 20 visualized in Figure 3A. The five most significant processes included the cellular response to organonitrogen compounds, cellular response to organic cyclic compounds, cellular response to nitrogen compounds, positive regulation of cell population proliferation, and cellular response to endogenous stimuli. For cellular components (CC), 129 elements were identified, and the top 20 are displayed in Figure 3B. The five most notable components identified include the spanning component of the plasma membrane, the integral component of the presynaptic membrane, the intrinsic component of the presynaptic membrane, caveola, and the integral component of the postsynaptic membrane. These results highlight the strong association of the targets with membrane-related functions essential for neuronal signaling. For molecular functions (MF), 153 elements were analyzed, with the top 20 shown in Figure 3C. The five most significant functions identified are Gq/11-coupled serotonin receptor activity, serotonin binding, amine binding, G protein-coupled serotonin receptor activity, and serotonin receptor



	Gens	Degree	Betweenness	Closeness
1	EGFR	31	1263.9622	0.5534591
2	PTGS2	29	1194.0514	0.54320985
3	ERBB2	28	627.8683	0.50285715
4	HTR1A	25	399.53726	0.4680851
5	PARP1	22	364.85806	0.46560848
6	MDM2	22	382.80444	0.4680851
7	DRD2	22	829.24817	0.5
8	SLC6A4	21	393.47287	0.4489796
9	HTR2A	20	173.23912	0.4467005
10	IGF1R	20	151.05225	0.460733

Fig. 2 **PPI Analysis. A. The extracted PPI network visualized using STRING, illustrating the interactions among key proteins. B. Analysis of the PPI network conducted with Cytoscape, showcasing the relationships and connectivity of the proteins involved. C. A subnet depicting the top ten genes identified in the analysis, highlighting their central roles within the network. D. A table summarizing the top ten genes along with their respective values for degree, betweenness, and closeness centrality, providing insights into their significance in the context of ML and AD.**

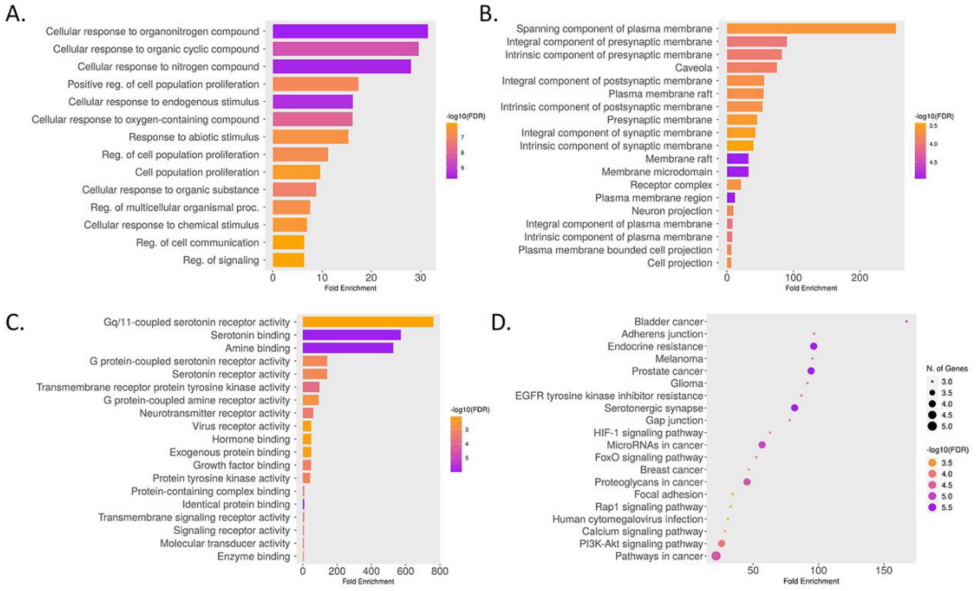


Fig. 3 **Enrichment Analysis of GO and KEGG Pathways. A. Biological Processes (BP) highlighting the significant roles of the identified targets. B. Cellular Components (CC) illustrating the structural contexts in which these targets operate. C. Molecular Functions (MF) detailing the biochemical activities associated with the targets. D. KEGG Pathway Analysis showcasing the top pathways enriched among the identified genes, emphasizing their potential involvement in various biological processes and disease mechanisms.**

activity. These findings emphasize the role of the targets in neurotransmitter signaling pathways, particularly those involving serotonin. Furthermore, the KEGG pathway enrichment analysis identified 97 pathways, with the top 20 visualized in Figure 3D. The five most significant pathways were bladder cancer, adherens junction, endocrine resistance, melanoma, and prostate cancer. These findings offer valuable insights into the potential involvement of the identified targets in various disease contexts, highlighting their importance in understanding ML's effects on AD.

Molecular Docking

Molecular docking was performed to assess the binding affinity of ML with five key proteins associated with AD. The docking scores revealed the following affinities: EGFR (–6.3), PTGS2 (COX-2) (–8.0), ERBB2 (–8.3), HTR1A (–7.0), and PARP1 (–7.5). Among these, ERBB2 exhibited the strongest binding affinity with ML, suggesting it as the most promising therapeutic target for AD. The negative docking scores indicate favorable interactions, with lower values corresponding to stronger affinities. These findings emphasize the potential of ML as a therapeutic agent for Alzheimer's disease, particularly through its interaction with ERBB2, which may play a pivotal role in modulating the disease's progression and associated neurodegeneration (Figure 4).

Discussion

Our objective in conducting this study was to utilize network pharmacology to explore and identify the complex interactions between ML and AD through the integration of bioinformatics and systems biology. By analyzing the multi-target and multi-channel mechanisms of ML in AD, we aimed to uncover its therapeutic potential, enhance our understanding of its molecular actions, and identify key targets that could inform the development of more effective treatments for AD. Additionally, we sought to evaluate the pharmacokinetic properties and safety profile of ML, assessing its suitability as a potential multi-target therapeutic agent for AD.AD, the leading cause of senile dementia,[30] presents increasing challenges due to its rising prevalence, highlighting the limitations of single-target drugs and the potential benefits of multitarget approaches like ML. This condition is a gradual process that unfolds over decades, beginning with amyloid deposition and progressing to tau pathology, brain atrophy, and synaptic dysfunction, ultimately leading to cognitive decline, memory loss, and behavioral changes.[31] In AD, the accumulation of Aβ and inflammation disrupts the integrity of the BBB, increasing levels of reactive oxygen species (ROS), matrix metalloproteinase-2 (MMP-2), and interferon-gamma (IFNγ). These changes facilitate the entry of neurotoxins into the brain, exacerbating disease progression.[32]

ML, the main hormone secreted by the pineal gland, is essential for regulating circadian rhythms and has been demonstrated to affect a range of physiological processes.[33] This makes it a potential therapeutic candidate for AD, where circadian disruptions are commonly observed. ML treatment has demonstrated promise in improving cognitive function and alleviating sleeplessness in neurodegenerative diseases by inhibiting apoptosis, activating the Nrf2 pathway and suppressing pro-inflammatory cytokines.[34,35] Chronic exposure to ML may mitigate these effects by reducing tau protein hyperphosphorylation through the activation of the PI3K/Akt/GSK3β pathway, offering a multifaceted approach to AD management.[36] The molecular characteristics and physicochemical properties of ML suggest excellent drugability, with one of its most significant features being its high permeability into the brain.[37] ML easily crosses the BBB, accumulating in the CNS at concentrations significantly higher than those found in the bloodstream.[38]

In this study, we assessed the pharmacokinetic properties and toxicity of ML using electronic databases, with the predicted LD50 estimated at 963 mg/kg, suggesting that ML is relatively safe. However, caution is advised regarding its potential effects on the nervous and respiratory systems. The findings showed that ML is non-toxic at doses up to 250 mg/kg and is

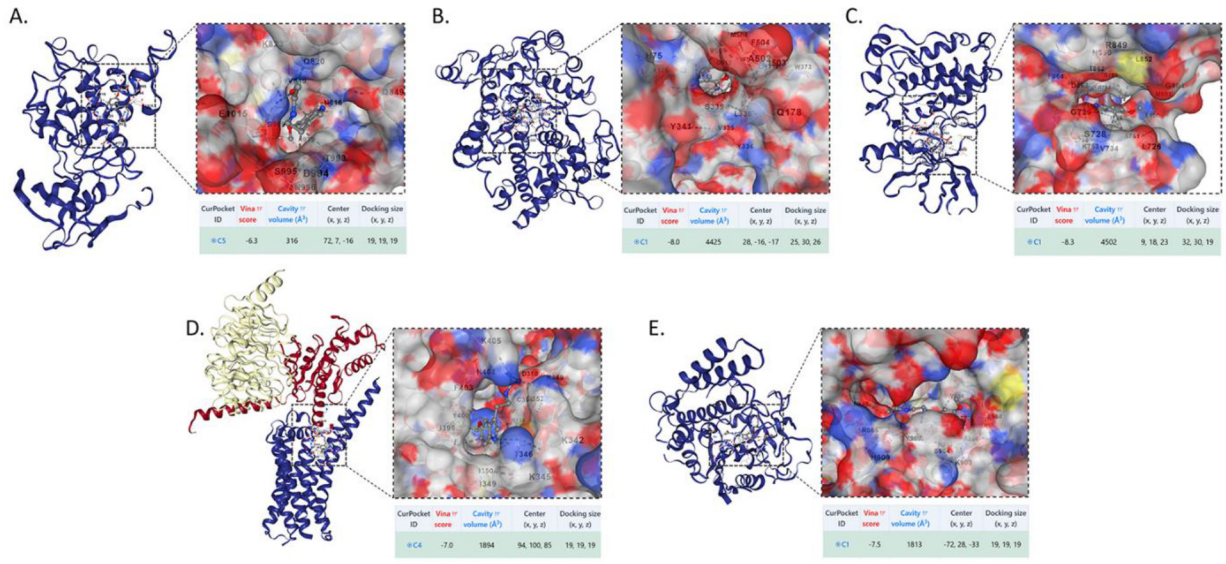


Fig. 4 **Molecular docking analysis of ML with five key target proteins for the treatment of AD. A. EGFR (score: -6.3), B. PTGS2 (score: -8.0), C. ERBB2 (score: -8.3), D. HTR1A (score: -7.0), and E. PARP1 (score: -7.5). The docking scores indicate the binding affinities of ML, with ERBB2 showing the strongest interaction, suggesting its potential as a therapeutic target in AD management.**

effective in shielding mice from the fatal consequences of acute whole-body irradiation.[39] Additionally, ML demonstrated significant safety, with an LD50 of 1,131 mg/kg in mice and 1,168 mg/kg in rats after intraperitoneal injection. This confirms its low toxicity even at high doses,[40] which aligns closely with the predicted results from the electronic databases.

We identified 15,564 genes associated with AD and 101 targets related to ML, with 95 common targets shared between ML and AD, as visualized through network analysis. The analysis of the ML-AD PPI network revealed the top 10 targets based on degree centrality: EGFR, PTGS2, ERBB2, HTR1A, PARP1, MDM2, DRD2, SLC6A4, HTR2A, and IGF1R, with ERBB2 emerging as the highest-ranking target. GO annotation and KEGG pathway analysis identified five highly significant pathways: bladder cancer, adherens junction, endocrine resistance, melanoma, and prostate cancer, as shown in Figures 3C-D. These pathways underscore the diverse roles of the identified targets in various disease contexts, highlighting their potential relevance in elucidating the mechanisms underlying ML's effects on AD.

In the context of these findings, Wang et al. (2024) demonstrated that ML enhances anti-glioma efficacy by inhibiting EGFR phosphorylation and dimerization, which may improve treatment outcomes in glioma patients.[41] Additionally, Choi et al. (2023) found that EGFR levels are increased in animal models of AD as well as in AD patients, contributing to memory impairment, astrogliosis, neuroinflammation, and the development of Aβ plaques.[42] Additionally, PTGS2, also known as COX-2, plays a significant role in the pathogenesis of AD by contributing to inflammation and being associated with genetic polymorphisms that increase the risk of developing the disease.[43] Yao and colleagues demonstrated that ML inhibits PTGS2 expression in lipopolysaccharide (LPS)-induced microglia by blocking the MAPK/NF-κB signaling pathway, thereby reducing inflammation.[44]

Furthermore, the ML derivative 6a (MD6a) inhibits PARP-1, leading to a reduction in mTOR/heat shock factor 1 signaling, which enhances mitochondrial function and protein homeostasis in Parkinson's disease models.[45] Recent studies have highlighted the crucial role of PARP1 in neurodegenerative diseases and aging by linking stress signals from neuroinflammation, mitochondrial dysfunction, and autophagy dysregulation.[46]

The atypical buildup of MDM2 in tau tangles of Alzheimer's suggests that the Tau/MDM2/p53 axis plays a role in both cancer and neurodegenerative processes.[47] Importantly, ML boosts p53 acetylation and activity by regulating the MDM2/MDMX/p300 pathway, which lowers MDM2 levels, prevents its nuclear translocation, and elevates p21 expression, thereby promoting cell growth inhibition and apoptosis.[48] The DRD2 gene, which is strongly linked to sleep duration,[49] exhibits a decline in the frequency of the A1 allele as individuals age, indicating an age-related modulation of DRD2's involvement in disease associations.[50] Additionally, SLC6A4 polymorphisms play a key role in depression and social cognition, with implications for age-related depressive symptoms; ML may influence its expression, potentially modulating neurodegenerative processes such as AD.[51]

The Htr1a and Htr2a genes, which play a role in serotonin signaling, show increased mRNA expression in the hippocampus of OXYS rats at the onset of Alzheimer's disease symptoms, suggesting their involvement in the neuroinflammatory and neurodegenerative processes linked to AD.[52] IGF-1 is essential for neurodevelopment and survival, and ML has been found to increase IGF-1 levels, potentially alleviating behavioral, neurochemical, and histopathological abnormalities in experimental models of obsessive-compulsive disorder.[53] Our study identified ErbB2 as the top-ranking target in relation to both Alzheimer's disease and ML (Figure 4). The Human Epidermal Growth Factor Receptor 2 (HER2 or ErbB2) is a type I transmembrane receptor tyrosine kinase, part of the EGFR family.[54] Interestingly, HER2 does not directly interact with ligands but operates through homodimerization or heterodimerization with other members of the HER/ErbB family, activating its tyrosine kinase domain.[55] This activation initiates downstream signaling pathways, such as the Ras-MAPK/ERK cascade, which is essential for cell proliferation and survival.[56]

The overexpression of HER2 on the cell membrane is a defining feature of a clinically aggressive and metastatic form of breast cancer, referred to as HER2-positive breast cancer. This subtype frequently involves ERK-mediated transcriptional regulation through the phosphorylation of transcription factors such as CREB, STAT3, p53, and JUN, which play a role in tumorigenesis and cancer progression.[57,58] In contrast, reduced ERBB2 levels are linked to mitochondrial dysfunction, stress responses—especially in cardiac function—and alterations in metabolic pathways.[59]

Changes in the mRNA expression of cytokines such as IL-6, TNFα, leptin, adiponectin, C-reactive protein (CRP), and tumor markers like TP53 and ERBB2 in peripheral blood are closely associated with the progression and pathophysiology of breast cancer.[60] Recent research has discovered EGFR as a new binding target of ML, which inhibits EGFR phosphorylation in both endometriotic cell lines and mouse models. This suppression affects the PI3K-Akt signaling pathway and halts the cell cycle by reducing Cyclin D1 (CCND1) expression.[61] The EGFR/ErbB signaling pathway has been closely associated with the pathogenesis of AD, as the activation of Ras GTPase by EGF stimulates γ-secretase activity, enhancing the nuclear function of the APP intracellular domain (AICD). Furthermore, intracellular mediators of EGF signaling, including Grb2, ShcA, and Abl, interact directly or indirectly with APP, reinforcing the link between EGFR/ErbB signaling and AD susceptibility. These findings underscore the pivotal role of this pathway in the progression of AD.[62]

Conclusion

In conclusion, this study employed network pharmacology to investigate the mechanisms underlying ML's effects in AD, pinpointing ERBB2 as a crucial therapeutic target. The results indicate that ML can address AD pathology through a multifactorial, multitarget, and multipathway approach, providing essential insights to inform future clinical strategies and decision-making in AD treatment.

Conflict of Interest

None.

References

1. Reddy PH. A critical assessment of research on neurotransmitters in Alzheimer's disease. Journal of Alzheimer's Disease. 2017;57(4):969–74.
2. Bature F, Guinn B-a, Pang D, Pappas Y. Signs and symptoms preceding the diagnosis of Alzheimer's disease: a systematic scoping review of literature from 1937 to 2016. BMJ open. 2017;7(8):e015746.
3. Mahakizadeh S, Mokhtari T, Navaee F, Poorhassan M, Tajik A, Hassanzadeh G. Effects of chronic hypoxia on the expression of seladin-1/Tuj1 and the number of dark neurons of hippocampus. Journal of Chemical Neuroanatomy. 2020;104:101744.
4. Roy J, Tsui KC, Ng J, Fung M-L, Lim LW. Regulation of melatonin and neurotransmission in Alzheimer's disease. International Journal of Molecular Sciences. 2021;22(13):6841.
5. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. The Lancet Neurology. 2010;9(1):119–28.
6. Chen KH, Reese EA, Kim HW, Rapoport SI, Rao JS. Disturbed neurotransmitter transporter expression in Alzheimer's disease brain. Journal of Alzheimer's disease : JAD. 2011;26(4):755–66.
7. Ramalingam M, Kim S-J. Reactive oxygen/nitrogen species and their functional correlations in neurodegenerative diseases. Journal of Neural Transmission. 2012;119:891–910.
8. Olufunmilayo EO, Gerke-Duncan MB, Holsinger RD. Oxidative stress and antioxidants in neurodegenerative disorders. Antioxidants. 2023;12(2):517.
9. Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N. Neuroinflammation as a common feature of neurodegenerative disorders. Frontiers in pharmacology. 2019;10:1008.
10. Mokhtari T. Targeting autophagy and neuroinflammation pathways with plant-derived natural compounds as potential antidepressant agents. Phytotherapy Research. 2022;36(9):3470–89.
11. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: under promises but over delivers. Journal of pineal research. 2016;61(3):253–78.
12. Santos TCd, Gomes TM, Pinto BAS, Camara AL, Paes AMDA. Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy. Frontiers in Pharmacology. 2018;9:1192.
13. Kandimalla R, Reddy PH. Therapeutics of neurotransmitters in Alzheimer's disease. Journal of Alzheimer's Disease. 2017;57(4):1049–69.
14. Hedayatpour A, Shiasi M, Famitafreshi H, Abolhassani F, Ebrahimnia P, Mokhtari T, et al. Co-administration of progesterone and melatonin attenuates ischemia-induced hippocampal damage in rats. Journal of molecular neuroscience. 2018;66:251–60.
15. Mokhtari T, Yue L-P, Hu L. Exogenous melatonin alleviates neuropathic pain-induced affective disorders by suppressing NF-κB/NLRP3 pathway and apoptosis. Scientific reports. 2023;13(1):2111.
16. Azizi M, Pasbakhsh P, Nadji SA, Pourabdollah M, Mokhtari T, Sadr M, et al. Therapeutic effect of perinatal exogenous melatonin on behavioral and histopathological changes and antioxidative enzymes in neonate mouse model of cortical malformation. International Journal of Developmental Neuroscience. 2018;68:1–9.
17. Morgese MG, Trabace L. Monoaminergic system modulation in depression and Alzheimer's disease: a new standpoint? Frontiers in pharmacology. 2019;10:483.
18. Roy J, Tsui KC, Ng J, Fung ML, Lim LW. Regulation of Melatonin and Neurotransmission in Alzheimer's Disease. Int J Mol Sci. 2021;22(13).
19. Pandi-Perumal SR, Trakht I, Srinivasan V, Spence DW, Maestroni GJ, Zisapel N, et al. Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Progress in neurobiology. 2008;85(3):335–53.
20. Zhang H, Zhang Y, Li Y, Wang Y, Yan S, Xu S, et al. Bioinformatics and network pharmacology identify the therapeutic role and potential mechanism of melatonin in AD and rosacea. Frontiers in Immunology. 2021;12:756550.
21. Noor F, Tahir ul Qamar M, Ashfaq UA, Albutti A, Alwashmi ASS, Aljasir MA. Network pharmacology approach for medicinal plants: review and assessment. Pharmaceuticals. 2022;15(5):572.
22. Li L, Yang L, Yang L, He C, He Y, Chen L, et al. Network pharmacology: a bright guiding light on the way to explore the personalized precise medication of traditional Chinese medicine. Chinese Medicine. 2023;18(1):146.
23. Mokhtari T, Ayman EL. Molecular mechanisms of Schisandra chinensis in treating depression-neuropathic pain comorbidity by network pharmacology and molecular docking analysis. Neuroscience. 2024;555:92–105.
24. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports. 2017;7(1):42717.
25. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic acids research. 2018;46(W1):W257–W63.
26. Kohl M, Wiese S, Warscheid B. Cytoscape: software for visualization and analysis of biological networks. Data mining in proteomics: from standards to applications. 2011:291–303.
27. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic acids research. 2023;51(D1):D638–D46.
28. Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics. 2020;36(8):2628–9.
29. Liu Y, Yang X, Gan J, Chen S, Xiao Z-X, Cao Y. CB-Dock2: Improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic acids research. 2022;50(W1):W159–W64.
30. Chiquita S, Rodrigues-Neves AC, Baptista FI, Carecho R, Moreira PI, Castelo-Branco M, et al. The Retina as a Window or Mirror of the Brain Changes Detected in Alzheimer's Disease: Critical Aspects to Unravel. Molecular neurobiology. 2019;56(8):5416–35.
31. Mohammadi S, Zandi M, Dousti Kataj P, Karimi Zandi L. Chronic stress and Alzheimer's disease. Biotechnology and applied biochemistry. 2022;69(4):1451–8.
32. Shin Y, Choi SH, Kim E, Bylykbashi E, Kim JA, Chung S, et al. Blood-Brain Barrier Dysfunction in a 3D In Vitro Model of Alzheimer's Disease. Advanced science (Weinheim, Baden-Wurttemberg, Germany). 2019;6(20):1900962.
33. Mohammadi S, Zahmatkesh M, Asgari Y, Aminyavari S, Hassanzadeh G. Evaluation of hippocampal arylalkylamine N-acetyltransferase activity in amyloid-β neurotoxicity. Journal of molecular endocrinology. 2023;71(2).
34. Naseri S, Moghahi SMHN, Mokhtari T, Roghani M, Shirazi AR, Malek F, et al. Radio-protective effects of melatonin on subventricular zone in irradiated rat: decrease in apoptosis and upregulation of nestin. Journal of Molecular Neuroscience. 2017;63:198–205.
35. Das R, Balmik AA, Chinnathambi S. Melatonin Reduces GSK3β-Mediated Tau Phosphorylation, Enhances Nrf2 Nuclear Translocation and Anti-Inflammation. ASN neuro. 2020;12:1759091420981204.
36. Xu J, Gao H, Zhang L, Rong S, Yang W, Ma C, et al. Melatonin alleviates cognition impairment by antagonizing brain insulin resistance in aged rats fed a high-fat diet. Journal of pineal research. 2019;67(2):e12584.
37. Al-Zaqri N, Pooventhiran T, Alsalme A, Warad I, John AM, Thomas RJJoML. Structural and physico-chemical evaluation of melatonin and its solution-state excited properties, with emphasis on its binding with novel coronavirus proteins. 2020;318:114082.
38. Tan DX. Melatonin and brain. Current neuropharmacology. 2010;8(3):161.
39. Meltz ML, Reiter RJ, Herman TSJMRF, Mutagenesis MMo. Melatonin and protection from whole-body irradiation: survival studies in mice. 1999;425(1):21-7.
40. Sugden DJJoP, Therapeutics E. Psychopharmacological effects of melatonin in mouse and rat. 1983;227(3):587–91.
41. Wang F, Zhu Y, Wanggou S, Lin D, Su J, Li X, et al. A natural compound melatonin enhances the effects of Nimotuzumab via inhibiting EGFR in glioblastoma. Cancer letters. 2024;592:216920.
42. Choi HJ, Jeong YJ, Kim J, Hoe HS. EGFR is a potential dual molecular target for cancer and Alzheimer's disease. Frontiers in pharmacology. 2023;14:1238639.
43. Ma SL, Tang NL, Zhang YP, Ji LD, Tam CW, Lui VW, et al. Association of prostaglandin-endoperoxide synthase 2 (PTGS2) polymorphisms and Alzheimer's disease in Chinese. Neurobiology of aging. 2008;29(6):856–60.
44. Yao C, Liu X, Zhou Z, Xiang Y, Yuan S, Xie W, et al. Melatonin attenuates expression of cyclooxygenase-2 (COX-2) in activated microglia induced by lipopolysaccharide (LPS). Journal of toxicology and environmental health Part A. 2019;82(7):437–46.
45. Ma QW, Han RT, Wu ZJ, Zhou JJ, Chen MT, Zhang XZ, et al. Melatonin derivative 6a as a PARP-1 inhibitor for the treatment of Parkinson's disease. Frontiers in pharmacology. 2024;15:1363212.
46. Mao K, Zhang G. The role of PARP1 in neurodegenerative diseases and aging. The FEBS journal. 2022;289(8):2013–24.
47. Sola M, Rendon-Angel A, Rojo Martinez V, Sgrignani J, Magrin C, Piovesana E, et al. Tau protein binds to the P53 E3 ubiquitin ligase MDM2. Scientific reports. 2023;13(1):10208.

48. Proietti S, Cucina A, Dobrowolny G, D'Anselmi F, Dinicola S, Masiello MG, et al. Melatonin down-regulates MDM2 gene expression and enhances p53 acetylation in MCF-7 cells. Journal of pineal research. 2014;57(1):120–9.
49. Cade BE, Gottlieb DJ, Lauderdale DS, Bennett DA, Buchman AS, Buxbaum SG, et al. Common variants in DRD2 are associated with sleep duration: the CARe consortium. Human molecular genetics. 2016;25(1):167–79.
50. Small GW, Noble EP, Matsuyama SS, Jarvik LF, Komo S, Kaplan A, et al. D2 dopamine receptor A1 allele in Alzheimer disease and aging. Archives of neurology. 1997;54(3):281–5.
51. Ugartemendia L, Bravo R, Reuter M, Castaño MY, Plieger T, Zamoscik V, et al. SLC6A4 polymorphisms modulate the efficacy of a tryptophan-enriched diet on age-related depression and social cognition. Clinical Nutrition. 2021;40(4):1487–94.
52. Alsallum M, Kaminskaya YP, Tsybko A, Kolosova N, Naumenko VJAiG. Patterns of Expression of the Key Genes of the BDNF System and Serotonin Receptors in the Brain of OXYS Rats in the Development of the Signs of Alzheimer's Disease. 2023;13(2):84–93.
53. Giri A, Mehan S, Khan Z, Gupta GD, Narula AS. Melatonin-mediated IGF-1/GLP-1 activation in experimental OCD rats: Evidence from CSF, blood plasma, brain and in-silico investigations. Biochemical Pharmacology. 2023;217:115831.
54. Henderson IC, Patek AJ. The relationship between prognostic and predictive factors in the management of breast cancer. Breast cancer research and treatment. 1998;52(1–3):261–88.
55. Zhang Q, Park E, Kani K, Landgraf R. Functional isolation of activated and unilaterally phosphorylated heterodimers of ERBB2 and ERBB3 as scaffolds in ligand-dependent signaling. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(33):13237–42.
56. Treisman R. Regulation of transcription by MAP kinase cascades. Current opinion in cell biology. 1996;8(2):205–15.
57. Strelkov IS, Davie JR. Ser-10 phosphorylation of histone H3 and immediate early gene expression in oncogene-transformed mouse fibroblasts. Cancer research. 2002;62(1):75–8.
58. Smith JA, Poteet-Smith CE, Xu Y, Errington TM, Hecht SM, Lannigan DA. Identification of the first specific inhibitor of p90 ribosomal S6 kinase (RSK) reveals an unexpected role for RSK in cancer cell proliferation. Cancer research. 2005;65(3):1027–34.
59. Mao L, Summers W, Xiang S, Yuan L, Dauchy RT, Reynolds A, et al. Melatonin Represses Metastasis in Her2-Postive Human Breast Cancer Cells by Suppressing RSK2 Expression. Molecular cancer research : MCR. 2016;14(11):1159–69.
60. Alokail MS, Al-Daghri NM, Mohammed AK, Vanhoutte P, Alenad A. Increased TNF α, IL-6 and ErbB2 mRNA expression in peripheral blood leukocytes from breast cancer patients. Medical oncology (Northwood, London, England). 2014;31(8):38.
61. Li Y, Hung SW, Zheng X, Ding Y, Zhang T, Tan Z, et al. Melatonin Inhibits Endometriosis Growth via Specific Binding and Inhibition of EGFR Phosphorylation. J Pineal Res. 2024;76(8):e70022.
62. Wang BJ, Her GM, Hu MK, Chen YW, Tung YT, Wu PY, et al. ErbB2 regulates autophagic flux to modulate the proteostasis of APP-CTFs in Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(15):E3129–e38.