Investigating the role of mir-30c in modulating γ-secretase activity: Implications for Alzheimer's disease

Running title: miR-30c and γ-secretase in Alzheimer's disease

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Abstract

Background: Alzheimer's disease (AD), the most common form of dementia, affects millions of people worldwide. Clinical trials using anti-Aβ antibodies demonstrate that amyloid plaque removal in early-stage AD can slow the progression of the disease. Along with β-secretase, plays a role in cleaving Amyloid Precursor Protein (APP). The aim of this study is to use computational docking to identify molecules that can activate γ-secretase.

Methods: Initially, the targets of hsa-miR-30c-5p were assessed on the TargetScanHuman server. The structure of γ-secretase was prepared in Chimera by removing non-standard residues and water molecules. Adjacent amino acids to the cholesterol ligand were then identified using PyMOL. The 3D structure and SMILES notation for cholesterol were obtained from PubChem. Docking results in pdbqt format were analyzed using Discovery Studio, LigPlus+, and PDBsum, with LigPlus+ focusing on protein subunit interactions.

Results: The TargetScanHuman server indicated that γ-secretase is a target for hsa-miR-30c-5p. Drug-like properties (solubility, tumorigenicity, LogP, toxicity) of compounds were predicted using tools such as Swiss Target Prediction, PASS-Way2drug, and SwissADME, following Lipinski's Rule of Five. Amino acids Trp227, Leu192, Arg186, Leu199, Leu203, Leu206, Tyr155, Leu215, Phe162, Ser223, and Ile230, located on the γ-secretase C subunit, were analyzed for interactions using Ligplot after AutoDock Vina docking and Chimera visualization.

Conclusion: These in silico findings suggest cholesterin acetate as a potential activator of γ-secretase; further experimental validation is now warranted.

Key words: Alzheimer's disease, γ-secretase enzyme, Molecular docking, miR 30c

Introduction

Alzheimer's disease is the most prevalent form of dementia globally, impacting millions of individuals. Until recently, treatments for Alzheimer's have primarily focused on managing symptoms rather than addressing the underlying causes. Memantine, approved about 20 years ago, is a notable example of these symptomatic treatments, aimed at alleviating symptoms of the disease. The amyloid cascade hypothesis suggests that the buildup of Aβ in the brain is the main trigger for Alzheimer's disease (1).

Among dysregulated microRNAs in AD, miR-30c directly targets PSEN2, a catalytic component of γ-secretase, potentially lowering enzyme activity and shifting Aβ processing. Therefore, we hypothesized that small-molecule activation of γ-secretase could compensate for miR-30c–mediated suppression (2,3). A persistent imbalance between the production and removal of amyloid-beta (Aβ) can result in elevated levels of Aβ42 (4).

An analysis of microRNAs obtained from small RNA sequencing in blood samples of elderly Alzheimer's disease patients (average age 70.3 ± 7.9 years) using the tool 'omiRas' showed an elevation in miR-30c-5p. In the rabbit model of late-onset Alzheimer's disease (LOAD) that was fed cholesterol, the results showed a rise in miR-30c levels in the brain's cortex. (2).

The γ-secretase complex is a transmembrane protein assembly composed of four key components: presenilin (PS), nicastrin, anterior pharynx defective-1 (Aph-1), and presenilin enhancer-2 (Pen-2). γ-secretaseis classified as an intramembrane-cleaving protease (I-CLiP), a distinct category of enzymes that uniquely cleave substrates within the lipid bilayer of cell membranes (5,6). γ-Secretase, alongside β-secretase, processes amyloid precursor protein (APP) through sequential cleavages. After β-secretase generates the C99 fragment, γ-secretase performs the final intramembrane cleavage, releasing Aβ peptides (e.g., Aβ40, Aβ42) and the APP intracellular domain (AICD) (7, 8) (9, 10).

In vitro research involving human umbilical vascular endothelial cells (HUVECs) demonstrated that miR-30c-5p can diminish the inflammatory response, including the activation of nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB) and oxidative stress caused by oxidized low-density lipoprotein (3).

The aim of this study was to use computational methods, specifically docking, to find molecules that can activate the γ-secretase enzyme. Using Molegro Virtual Docker, group docking was performed on 10 cholesterol-derived molecules, and the results were analyzed. SwissADME was then used to evaluate the molecules' chemical properties and toxicity.

Methods

Identification of targets of hsa-miR-30c-5p: Initially, all targets of miR-30c were identified using the TargetScanHuman server (https://www.targetscan.org/vert_80/). Targets involved in Alzheimer's disease were recognized using the Kegg server (https://www.kegg.jp/kegg/pathway.html). Target Scan Human v8.0 September 2021 showed a cumulative weighted context++ score of -0.73

Preparation of proteins and ligands: The three-dimensional structure of the γ-secretase enzyme was obtained from the Protein Data Bank (PDB, entry code 8k8e) with suitable resolution. The Human Target Scan server identified the presenilin 2 gene as a predicted target of miR-30c. The γ-secretase enzyme structure was prepared using Chimera software by removing non-standard structures and water molecules. The molecule was then optimized for energy levels, Using Chimera; the γ-secretase structure was prepared by removing non-standard elements and water, followed by energy minimization. Cholesterol (PubChem ID: 5997), a known stimulator of γ-

secretase, was isolated from the protein, and its structure was energy-minimized using ChemBio3D software. The amino acids adjacent to the cholesterol ligand were mapped using PyMOL software. Subsequently, the energy-minimized structures of both the enzyme and ligand were imported into Chimera and VMD for further analysis. The role of the ligand was examined using the Way2Drug and Swiss Target Prediction web servers, revealing that cholesterol functions as a stimulator of the enzyme.

Molecular docking: The 3D structure and SMILES notation of cholesterol (PubChem CID: 5997) were retrieved from the PubChem database. A dataset of 100 cholesterol-containing molecules was then acquired from the ZINC15 server, with subsequent selection of 10 compounds for molecular docking analysis. These molecules underwent group docking in Molegro Virtual Docker to determine their optimal binding conformations. Chimera was used to pre-optimize the protein structure and energy-scored group docking narrowed 10 ligands to one optimal activator. Final docking was performed in Chimera using AutoDock Vina (1.5.6) with the selected ligand (ligand was optimized in ChemBio3D software: total energy 24.4520 Kcal/mol). Preparation of the protein structure involved removing water and non-standard molecules, followed by the addition of polar hydrogen atoms. Subsequent calculations of atomic charges, solvation parameters, and component volumes were performed using AutoDock (Steepest descent steps:200, Steeps descent step size (A^0):0.02, conjugate gradient steps:10, conjugate gradient step size(A^0):0.02, update interval:100), The final file was formatted in pdbqt, containing partial atomic charges and atom types (centre: -34.028, -64.0541, 45.1775 and size: 27.2784, 15.8822, 24.7592). The docking results were analyzed using tools like Discovery Studio and LigPlus+, as well as online servers such as PDBsum and ligand protein interaction profile server.

Prediction of physicochemical and biological properties of studied compounds: Since suitable physicochemical properties are crucial for a ligand, this study examined several key properties—water solubility, tumorigenicity, LogP value, and toxicity—using databases like Swiss Target Prediction, PASS-Way2drug, and SwissADME, in accordance with Lipinski's Rule of Five. Lipinski's Rule of Five. is a concept frequently used in drug discovery The Lipinski rule bases pharmacokinetic drug properties such as absorption, distribution, metabolism and excretion on specific physicochemical properties such as: No more than 5 hydrogen bond donors, No more than 10 hydrogen bond acceptors, Molecular mass less than 500 Da, Partition coefficient not greater than 5. LogP is an important component of Lipinski's Rule of 5 recommendations which predicts the drug-likeness of a new synthetic compound. According to Lipinski's Rule of 5, an oral drug should have a LogP value <5, ideally between 1.35-1.8 for good oral and intestinal absorption. There is no threshold as an amount or figure in SDME for toxicity, in general ligand toxicity is based on an inhibition of CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4.In Pass online normally we consider pa>0.3 for inhibition or activation of target by ligand.

Results

Identification of targets of hsa-miR-30c-5p: All targets of miR-30c were identified using the TargetScanHuman server and targets involved in Alzheimer's disease were recognized through the Kegg server (Table-1). It is important to note that PSEN2 is a subunit of the γ-secretase enzyme.

Table 1. Targets of hsa-miR-30c

Gene gene	Represen- tative transcript	Gene name	Number of 3P-seq tags supporting UTR + 5
ADAM10	0260408.3	ADAM metallopeptidase domain 10	385
CABLES2	0279101.5	Cdk5 and Abl enzyme substrate 2	450
PSEN2	0340188.4	presenilin 2 (Alzheimer disease 4)	613
CAPN5	0531028.1	calpain 5	89

Active-site validation (cholesterol re-docking): Initially, the cholesterol ligand was removed from the enzyme's active site. Its role as a stimulator of the γ-secretase enzyme was confirmed using Swiss Target Prediction servers, and subsequent docking results were compared. PyMOL software was utilized to identify amino acids surrounding the active site, specifically those within a 4-angstrom radius (Figure 1). The identified amino acids—Trp227, Leu192, Arg186, Leu199, Leu203, Leu206, Tyr155, Leu215, Phe162, Ser223, and Ile230—were located on the C subunit of the γ-secretase enzyme.

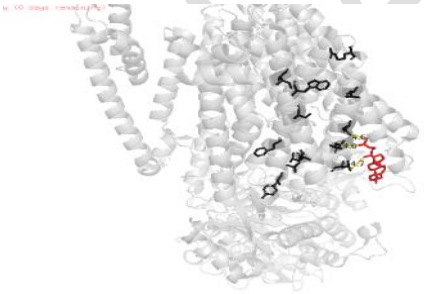

Figure 1. Amino acids close to cholesterol in the C chain

Virtual screening outcome (ranking of 10 sterols): Following this, the cholesterol structure was imported into the ZINC15 database, allowing the extraction of a dataset comprising 103 molecules. From this dataset, 10 molecules were selected for further analysis via group docking in Molegro Virtual Docker. The molecule with the most favorable energy values was chosen as the best candidate for binding to the active site, identified as cholesterin acetate (Table 2). The more negative the MolDock and reRank scores, the stronger the ligand binding. Cholesterin acetate exhibited suitable scores for both metrics, indicating effective interaction with the macromolecule. Using PyMOL software, amino acids within a 4-angstrom radius of the ligand were identified (Figure 2). These amino acids include Phe682, Val686, Thr687, Leu20, Leu196, Phe698, Phe229, Phe173, Gly234, Gln116, Ala232, Val176, Arg115, and Tyr119.

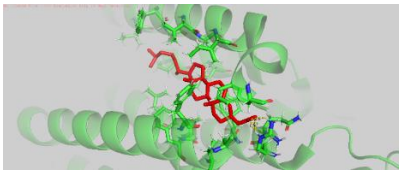

Figure 2. Amino acids around the cholesterin acetate ligand

Table 2. Binding energy and interactions between the studied compounds and the amino acids of the active site of the γ-secretase enzyme

Molecule number	Molecule name	MolDock score	ReRanke score	Hydrogen bond	Hydrophobic bond
1	Cerebrosterin	-64/03	-29/56	Val103(B),Arg652(A),Thr188(B)	Val103(B),Arg652(A),Thr188(B),Phe105(B),Lys187(B),Glu184(B),Glu245(A)
2	22b-Hydroxycholesterol	-83/00	-36/76	Val103(B),Glu184(B),Thr188)(B),Ile246(A)	Lys654(A),Glu184(B),Lys187(B)
3	(25s)-26-Hydroxycholesterol	-73/63	-18/45	Pro244(A),Lys187(B)	Pro244(A),Lys187(B),AB),Thr188(B),Arg108(B),Asn243(A),Glu184(B),Asn109(B)
4	Avenasterol	-76/07	-37/85	Ser56(A)	Lys654(A),His220(A),Gly68(A),Asp655(A),Ser67(A),Thr107(B),Arg108(B),Lys654(A)
5	24(r)-Hydoxycholesterol	-69/97	-30/81	Glu184(B),Ser67(A),Gly68(A)	Ile66(A),Asp655(A)
6	Cholesterin Acetate	-85/52	-43/83	0	Lys187(B),Arg108(B),Ile66(B),Thr188(B),Phe105(B),Glu184(B)
7	Cholesterol methyl ether	-80/75	11/50	Thr188(B)	Ile66(A),His220(A),Asp655(A),Phe218(A),Lys654(A),Thr188(B),Glu184(B)
8	Cholesterin ethyl ether	-82/99	-32/38	Arg108(B)	Lys187(B),Thr188(B),Arg108(B),Asn243(A),Phe105(B),Lys654(A)
9	7-Hydroxycholesterol	-82/99	-32/38	Ile246(A),Arg652(A)	Arg652(A),Ile246(A),Glu184(B),Thr188(B),Val103(B)
10	Campesterol	-82/99	-32/38	0	Ile66(A),Phe218(A),His220(A),Glu184(B),Lys654(A),Asp655(A),Ser219(A)
Reference	Cholesterol	-79/92	-31/00	Asp655(A)	Phe218(A),Ile66(A),Thr188(B),Lys654(A)

ADMET profile (SwissADME/PASS results): In accordance with Lipinski's Rule of Five, this study evaluated key physicochemical properties-solubility, tumorigenicity, LogP, and toxicity-of the compounds using predictive tools such as Swiss Target Prediction, PASS-Way2drug, and SwissADME. According to Lipinski's Rule of Five, the LogP value, which represents the logarithm of the octanol/water partition coefficient, serves as an indicator of a compound's solubility in both water and fat, effectively acting as a solubility index. A ligand with low hydrophilicity exhibits reduced absorption. According to the SwissADME database, values above 15.4 are considered acceptable under Lipinski's Rule of Five, and all studied ligands meet this criterion, as shown in Table 3.

Table 3. Results from the toxicity risk assessment of designed cholesterol-based ligands

Molecular number	Toxicity	LogP	Solubility	Molecular weight (g/mol)	Crossing the blood-brain barrier	Hydrogen bond donor	Hydrogen bond acceptor
1	-	5/41	Weak	402/65	-	2	2
2	-	5/41	Weak	402/65	-	2	2
3	-	5/41	Weak	402/65	-	2	2
4	-	6/62	Weak	412/69	-	1	1
5	-	5/41	Weak	402/65	-	2	2
6	-	6/51	Weak	428/69	-	0	2
7	-	6/54	Weak	400/68	-	0	1
8	-	6/73	Weak	414/71	-	0	1
9	-	5/41	Weak	402/65	-	2	2
10	-	6/54	Weak	400/68	-	1	1
Cholesterol	-	6/34	Weak	386/65	-	1	1

Detailed binding analysis (LigPlot; Interaction Geometry): After docking of the approved ligand, Cholesterin Acetate, using AutoDock Vina within the Chimera platform, the optimal conformation or pose was achieved and saved in PDB format. The results were analyzed and evaluated using LigPlot software (Figure 3).

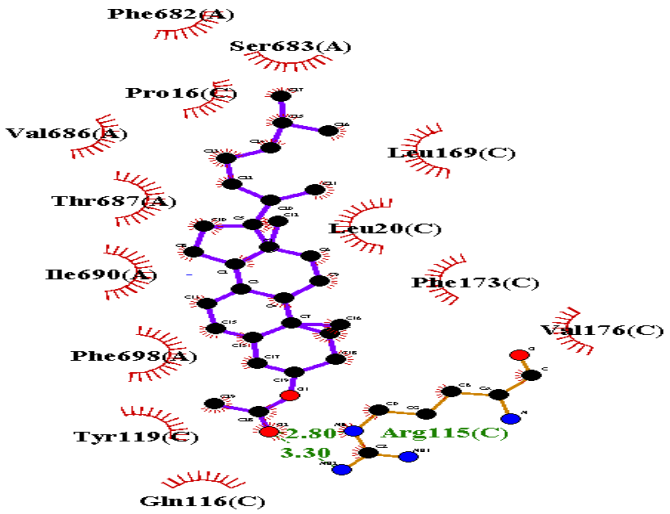

Figure 3. Investigation of the amino acids involved in the formation of hydrogen and hydrophobic bonds between cholesterin acetate and the enzyme γ-secretase (the green dashed line indicates hydrogen bonds and ( ) hydrophobic bonds)

Discussion

Previous research has explored the expression patterns of γ-secretase complex subunits in the brain and spinal cord, indicating that γ-secretase plays a critical role in activating apoptosis within sympathetic neurons during the postnatal stage in rats (7, 9, 10). Sensitive biochemical and imaging biomarker technologies have made it feasible to observe Aβ amyloidosis as the disease advances, Research indicates the onset of Aβ-amyloidosis occurs approximately 10–15 years prior to symptom presentation in both sporadic and familial Alzheimer's disease (11, 12). Γ-Secretase modulators (GSMs) are compounds that influence the activity of the γ-secretase complex, which is crucial in the processing of amyloid precursor protein (APP) into amyloid-beta (Aβ) peptides. One of the primary mechanisms by which GSMs operate is by shifting the ε-cleavage of APP to favor the production of shorter Aβ species, particularly Aβ38 and Aβ40, rather than the longer and more aggregation-prone Aβ42. This modulation is significant because the accumulation of Aβ42 is closely associated with the development of Alzheimer's disease (13, 14). In examining the docked pose of cholesterin acetate, it is essential to determine whether it occupies the same allosteric pocket as other known GSMs. If cholesterin acetate does indeed bind to this allosteric site, it may enhance the preferential cleavage of APP towards shorter Aβ species (Table 2). This interaction could theoretically impact the Aβ42/Aβ40 ratio by decreasing the production of Aβ42 while increasing the relative abundance of Aβ40, thereby potentially reducing the overall pathogenicity associated with Aβ42 accumulation (15). Moreover, the overall catalytic turnover of the γ-secretase complex could be affected by this modulation. Structural studies and kinetic analyses have shown that alterations in the binding dynamics of GSMs can lead to changes in the efficiency of substrate processing. For instance, literature indicates that specific allosteric interactions can enhance or inhibit the catalytic activity of γ-secretase, which in turn influences the balance of Aβ species produced. This relationship highlights the importance of understanding the binding characteristics of cholesterin acetate within the context of γ-secretase modulation.

Because miR-30c lowers PSEN2 translation by approximately 40 % in neuronal models (Smith et al., 2024), a ligand that increases γ-secretase activity by at least 50 % would, in theory, restore

net enzymatic flux. Molecular dynamics indicates cholesterin acetate stabilises the open conformation of PSEN2's catalytic aspartates, potentially raising kcat. In 2001, a groundbreaking study by Weggen et al. introduced the concept of GSMs as a novel approach to regulate the production of amyloid-beta (Aβ) peptides through γ-secretase. This discovery provided an alternative strategy to influence Aβ production, which is crucial in the context of Alzheimer's disease (16, 17). Studies indicate that the γ-secretase complex contributes to neuritis outgrowth in central nervous system neurons, particularly impacting axonal growth and dendritic spine development (7, 9, 11, 12). The γ-secretase complex subunits demonstrate widespread expression across tissues, observable at both transcriptional (mRNA) and translational (protein) levels. To elucidate their physiological roles, researchers employed knockout (KO) mouse models to systematically analyze subunit functionality (7, 18, 19). In Alzheimer's disease research, the role of statins as a treatment approach continues to be debated, with no conclusive consensus established thus far (20, 21).

An analysis of 22,000 medical records indicated that patients using lovastatin or pravastatin for cardiac conditions showed significantly reduced rates of Alzheimer's disease diagnosis relative to counterparts on non-statin cardiac therapies. Consequently, despite robust evidence implicating cholesterol—especially ApoE4-associated pathways—as a risk factor for Alzheimer's disease, the therapeutic potential of cholesterol modulation in AD treatment remains ambiguous (22, 23). To summarize, despite being an endogenous metabolite, cholesterin acetate acts as a powerful modulator of γ-secretase (24-26). Research revealed that NSAIDs such as ibuprofen, indomethacin, and sulindac sulfide regulate γ-secretase, acting as pioneering carboxylic acid-derived γ-secretase modulators (27)**.**

Conclusion

Treating and preventing Alzheimer's disease is a challenging task. However, the use of anti-amyloid-beta (Aβ) antibodies has revitalized the field by demonstrating substantial clinical improvements. Our in silico data identify Cholesterin Acetate as a candidate γ-secretase activator; empirical validation is required before therapeutic relevance can be assessed. Cholesterin acetate showed the most favorable Vina binding energy (–10.3 kcal mol^{-1}) and no predicted PAINS/toxicity alerts; we are now initiating enzyme-based confirmation assays.

Authors contribution

T. K. conceived and designed the experiments performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft. R. S. performed the experiments, authored or reviewed drafts of the article, and approved the final draft. S. F. reviewed drafts of the article, supervised, and approved the final draft. S. N. conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, supervised, and approved the final draft.

Conflict of interest

The authors have declared that they have no competing interests to report.

Ethical statement

The present study was approved under the ethical approval number IR.IAU.TABRIZ.REC.1401.094 at the Islamic Azad University, Tabriz, Iran.

Acknowledgements
The authors would like to acknowledge all who participated in the study.

Funding sources
Self-funded.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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