Apaf-1 as a Potent Drug Target for Neurodegenerative Diseases

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Abstract: *Neurodegeneration is a huge socioeconomic and humanistic problem which occurs as a result of neuronal cell death i.e. Apoptosis, which involves the activation of caspase enzyme by Apaf-1 in the presence of Cytochrome c and dATP. Recruitment of procaspase 9 to Apaf-1 is mediated by the presence of Caspase activation and recruitment domains (CARDs). In order to block or inhibit the apoptotic pathway that occurs during the neurodegenerative diseases, the interaction between Apaf-1 CARD and procaspase 9 was exploited. These findings that blocking the caspase activation and recruitment domain will result in Neuro-protection, helped in the designing of potent drugs that competitively interact with Apaf-1 CARD and do not allow the cleavage of procaspase 9 to yield its biologically active form i.e. caspase 9. Thereby no neuronal degradation will occur. The spontaneous interaction between the receptor - Apaf-1 and its natural ligand- procaspase 9 and also its interaction with its synthetic ligand, which can be exploited as the potent drug for neurodegeneration, were analyzed using several bioinformatics systems. Therefore, in the coming years, the compound can be exploited as a potent drug in order to obtain a reliable therapy for neurodegenerative diseases.*

Keywords: *Neurodegeneration, Apaf-1, CARD, Caspases, dATP.*

1. INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder which is a huge socioeconomic and humanistic problem [1]. It is considered to be a global epidemic even though the cases are disproportionately concentrated. The clear majority (62%) of people with dementia live in low and middle income countries where access to social protection, services, support and care are very limited [2] In the next few decades, the global burden of dementia will shift inexorably to poorer countries, particularly rapidly developing middle income countries. The severity of the Alzheimer's disease (AD) problem in the US is indicated by recent statistics from the Alzheimer's Association [3]. Approximately 5.4 million Americans are currently diagnosed with AD, including one in eight individuals aged 65 and older. By 2025 the number of Americans aged 65 and older with AD is expected to reach 6.7 million. Payments for health care, long-term care, and hospitals for AD patients, which are currently estimated at $200 billion, are expected to be $1.1 trillion (in US dollars) by 2050 [4]

Neuro-degeneration is due to neuronal cell death (Apoptosis). Apoptosis is a form of cell death in which a programmed sequence of genetically directed events lead to elimination/abolition /extrusion of cells essential for tissue homeostasis and development. It is strongly regulated by protein-protein complex formation and deregulation of it results in a number of human pathologies including neurodegenerative diseases [5].Apoptosis involves the activation of a group of cysteine proteases, Caspases, specific for aspartic acid residues [6].

The activation of neurodegeneration in Alzheimer's and Parkinson's is via activation of caspases through the apoptosome complex formation [7]. The apoptosome complex formation involves the release of pro-apoptotic factor, cytochrome c, from mitochondria and it's binding to Apaf-1 in the presence of ATP/dATP [8].

Apaf-1 has three functional domains – short N- terminal CARD (caspase activation and recruitment domain), central CED-4 homology domain and long WD-40 repeat domain [9]. Binding of cytochrome c to Apaf-1 exposes the CARD, which then interacts with the corresponding motif in the pro-domain of procaspase-9 resulting in the activation of apoptotic

pathway [10].Recruitment of procaspase-9 by Apaf-1 is central to its subsequent activation as disruption of this process results in the complete inhibition of procaspase-9 processing in a cell-free systems raising the possibility of using small molecules to intervene in apoptotic pathways [11]. The 97-residue CARD domain of Apaf-1 shares 20% sequence identity with the pro-domain of procaspase-9, which also belongs to the CARD family of apoptotic signaling motifs that allows it to bind selectively to the CARD in Apaf-1 through homotypic interactions. [12].
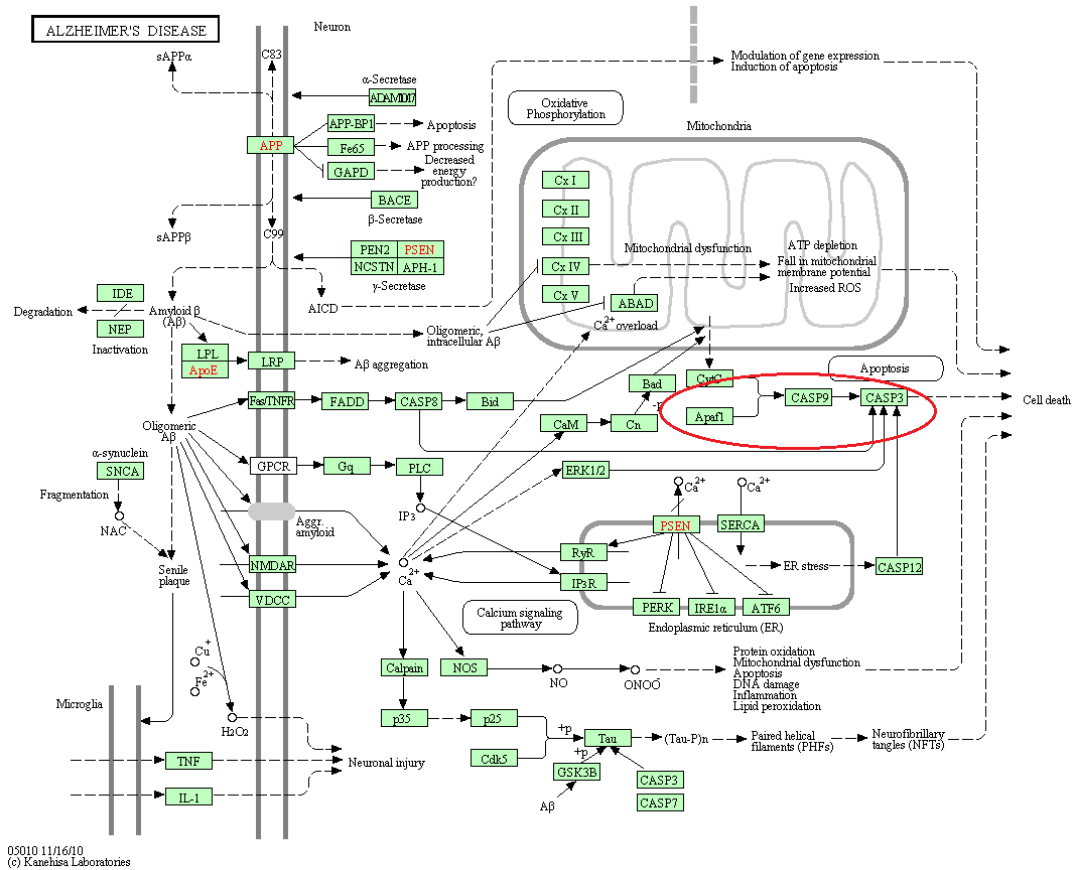
(a)
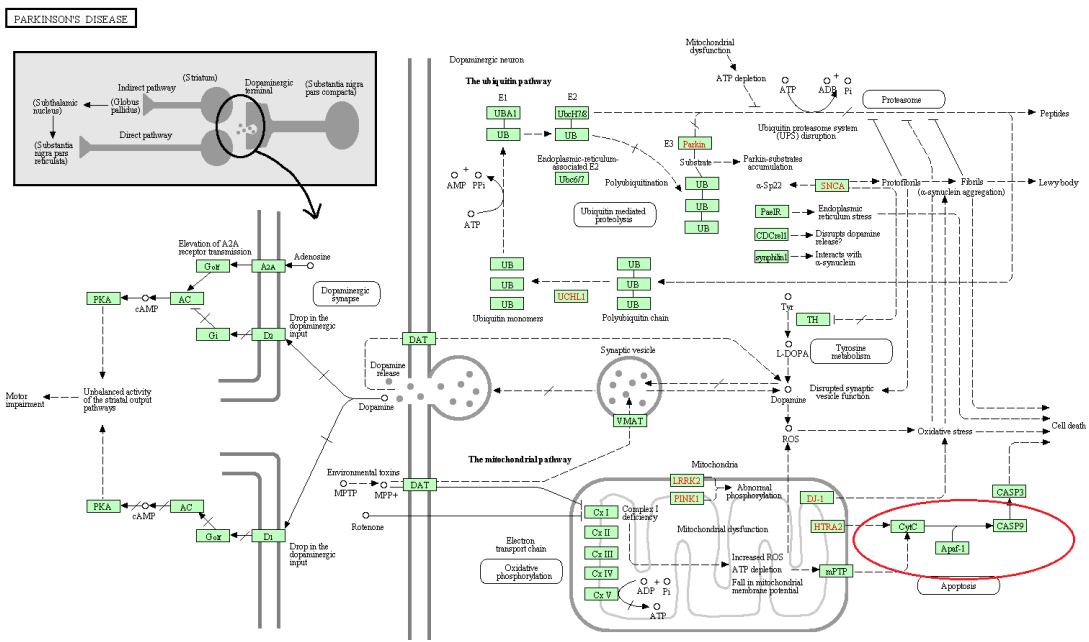
(b)

Fig1. *The KEGG pathway chart analysis of (a) Alzheimer's disease and (b) Parkinson's disease in order to identify the possible common point between the two.*

The study revealed that Apaf-1 is the common link between many neurodegenerative diseases like Alzheimer's and Parkinson's which was validated by KEGG pathways [13, 14] and also by Alibaba software for protein interaction (Fig. 1) [15]. Hence it was concluded that Apaf-1 protein can serve as a potent drug target to reduce apoptosis in neurodegenerative diseases. It is also known form the literature that dATP is a know ligand which binds to the protein in order to stabilize its interaction with the cytochrome c, further leading to caspases activation [16]. Therefore we have designed an inhibitor which will competitively inhibit the interaction of Apaf-1 with procaspase 9 and hence can act as a potent lead compound for such diseases.

2. MATERIALS AND METHODS

2.1. Protein Interaction Study

2.1.1. KEGG Pathways

Pathway maps of Alzheimer's and Parkinson's disease were procured for the biological interpretation of high-level systemic functions from the **KEGG PATHWAY.** It is a collection of manually drawn pathway maps representing our knowledge on the molecular interaction and reaction networks for: Metabolism, Genetic Information processing, environmental processing, cellular processes, organism systems, human disease and drug development. The map was used to study and find out the possible common protein link between the two diseases.

2.1.2. AliBaba Software

Ali Baba was used to search and visualize, protein and disease centered information from PubMed [17]. It displays the search results in the form of a graph, biological/medical objects such as proteins, diseases, or drugs are nodes; meaningful associations between them are edges. It was further used to check the interacting proteins, genes with implications in diseases and tissue specificity of genes.

2.2. Retrieval of Three Dimensional Structures of Apaf-1 CARD Domain Alone and its Complex with Procaspase 9 Respectively

The Three dimensional structures of Apaf1 CARD domain (PDB ID: 2P1H) and CARD domain - procaspase 9 complex (PDB ID: 3YGS) were procured from the RCSB PDB [18], database of all the available protein three dimensional structures.

2.3. Inhibitor Designing

The inhibitors were designed by ChemSketch software (Fig. 2) [19] and the mol. file obtained was later converted to the pdb. file in order to perform the docking via the OpenBable [20, 21] software. Also, the three dimensional structure of dATP was procured from PubChem database in sdf format (3D) which was then converted into a pdb file by the help of an online server: Online SMILES Translator and Structure File Generator (*http://cactus.nci.nih.gov/translate/*) and Open Babel: An open chemical toolbox.

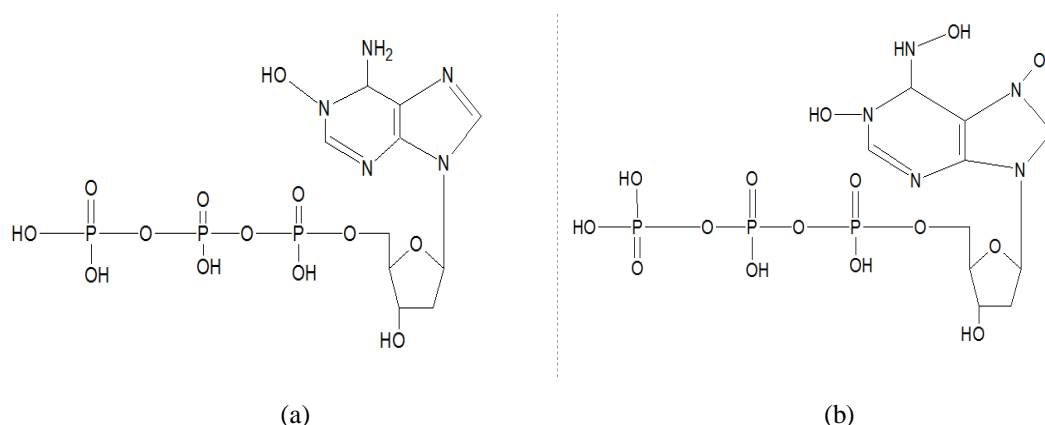(a)   (b)

Fig2. *The structure of (a) compound 1 and (b) compound 2*

2.4. Docking Study

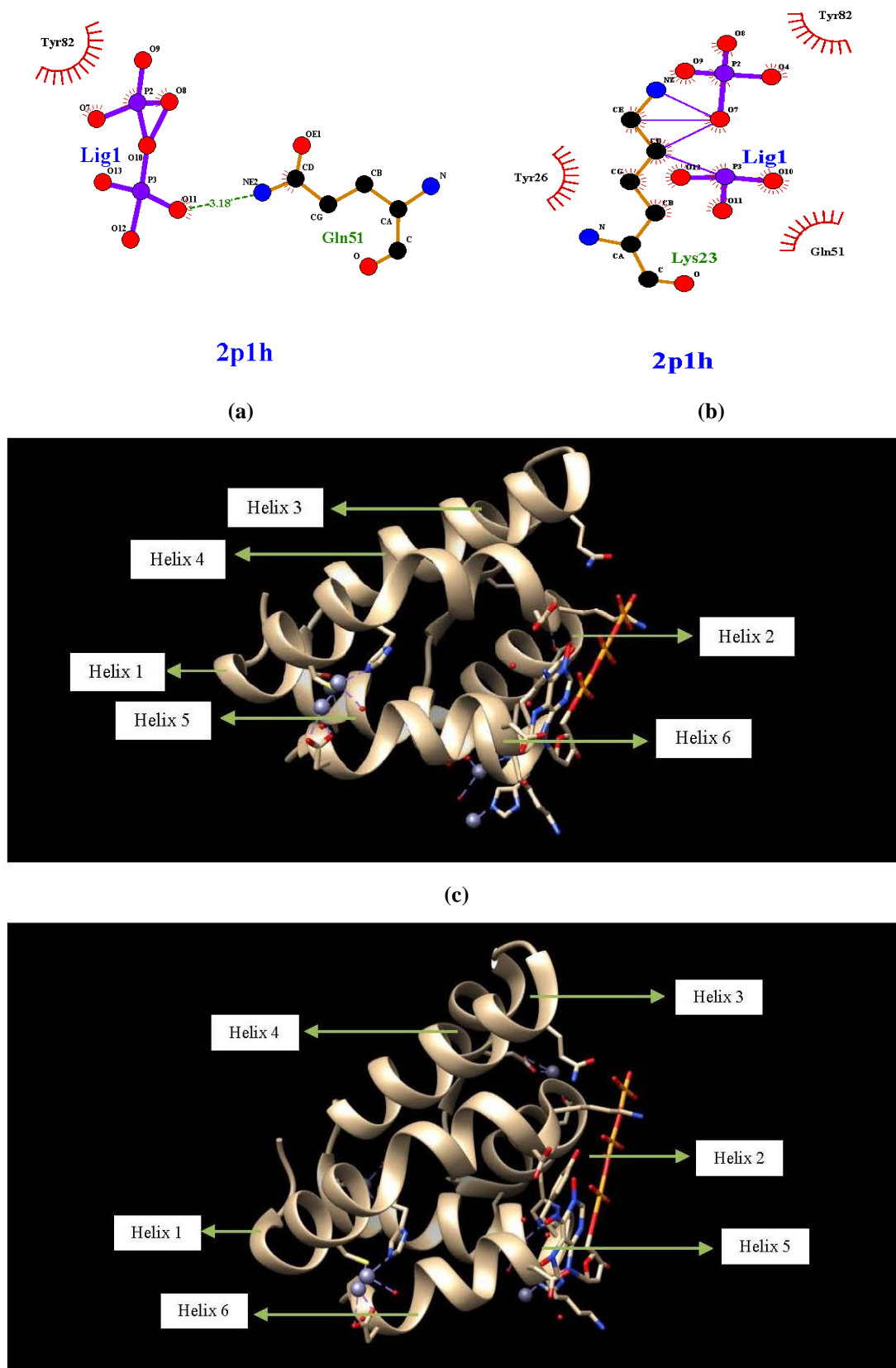Docking of Apaf-1 with different chemical ligands was done with the help of PatchDock, an online tool for ligand protein docking (Fig. 3) [22, 23] (http://bioinfo3d.cs.tau.ac.il/PatchDock).

(a)   **(b)**

(c)

Fig3. *Docking interaction between Apaf1 CARD domain and compound 1(a & d) and compound 2 (b & c) visualized by LigPlot Plus (a & b), chimera (c & d) and docking was done using PatchDock server.*

Table1. *The following table shows the interacting amino acid between Apaf-1 and the ligand as well as their docking scores via PatchDock.*

Ligand	Docking Score	Interacting Amino Acids
Compound 1	4140	Gln 51, Tyr82
Compound 2	4428	**Lys 23, Tyr 26**, Gln 51, Tyr 82

3. RESULT

The pathway map of Alzheimer's and Parkinson procured from the KEGG pathways provided with a common protein i.e. Apaf1 which was later validated via Alibaba Software, a software for the graphical representation of biological data allowing easier interpretation of the data from PubMed. The pdb file of Apaf-1 CARD (PDB ID: 2P1H) was procured and further used for the docking study with the different inhibitors. The docking score and the interacting amino acid residues were analyzed for all the interactions (Table 1) which showed very high docking score of 4140 of compound 1 and 4428 of compound 2 according to the PatchDock server. Also the interacting amino acid residues, analyzed by Chimera and LigPlot were found to be Gln51 and Tyr82 in case of compound 1 and Lys23, Tyr26, Gln51 and Tyr82 in case of compound 2.

4. DISCUSSION

The interaction of the drug with Apaf-1 CARD domain was studied and it was observed that the drug interacts at the same site, where the procaspase 9 interacts, with very high affinity. Hence it can be concluded that the drug is capable of competitively inhibiting the binding of procaspase 9 with Apaf-1 CARD. It should be noticed that the interacting amino acids between the drug (compound 2) and Apaf-1 CARD domain are Lys 23, Tyr 26, Gln 51 and Tyr 82, similar to the binding site of the pro-caspase 9 i.e. Asp 27, Ser 31 and Glu 40 [11]. The interaction between the drug and the CARD domain was calculated to be very high indicating an obvious competition between the drug and pro-caspases to bind at the same site showing that the drug will competitively inhibit the binding between CARD and pro-caspase9, therefore resulting in the inhibition of apoptosis of neuronal cells. Our findings were in consensus with the already published data, that is, the binding of pro-caspase 9 to the CARD domain of Apaf-1 (PDB ID: 3YGS) is of utmost importance for the activation of apoptosis [11].

Afpa1 is a potent target for the treatment of neurodegenerative disease like Alzheimer's and Parkinson's disease. The drug if targeted to the brain cells will inhibit the activation of the caspases and therefore, apoptosis of the neuronal cells. The molecular size of the drug is very small, making its targeting to the respective region easier and enabling it to cross BBB. The small size of the compound can also help in its easy linkage to the carrier molecule**.**

Therefore, it can be concluded that the drug can be used as a potent molecule for further research, in order to obtain a reliable medicine to reduce or terminate the damage in the patients suffering from neurodegenerative disorders.

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