Alzheimer's Disease & Treatment

Chapter 5

Drug Targets and Therapeutic Approaches of Alzheimer's Disease

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Abstract

Dementia is an emerging global clinical complication and it is associated with a variety of distinct pathological and intellectual functions. Alzheimer's disease (AD) is major part (70%–80%) of the dementia. AD is a progressive multifarious neurodegenerative disorder which affects the routine life of the patients with several complications like memory loss and difficulty in communication skills. The pathophysiological conditions of AD are influenced by a variety of genetic and environmental factors. Effective management of AD and other types of dementia is essential for better healthy longevity of patients. In the past two decades, advances in the field of pathogenesis have enthused the research community for investigation of novel pharmacological therapeutics with known targets of the disease. Currently available therapeutic methods are targeted indirectly, like acetylcholinesterase inhibitors and N-methyl d-aspartate receptor antagonist, which contribute to a minimal impact on the disease. The identification of a number of new targets and specific small molecules is also essential for the significant therapeutic management of AD. In this study, we report on the potential targets and recent developments in the discovery of inhibitors against the disease. Brief in silico studies are also included, to address the possibility of a theoretical lead in the drug designing for AD.

1. Introduction and Epidemiology of Alzheimer's Disease

Alzheimer's is a neurological disorder in which the death of brain cells causes memory loss and cognitive decline. It is a type of dementia, accounting for 60%–80% of cases of dementia in the world population. Alzheimer forms a major part of dementia, and is caused by genetic and environmental factors. In 2013, 6.8 million people in the US were diagnosed with dementia. Of these, 5 million were diagnosed with Alzheimer's Disease (AD). By 2050, the diagnosis of AD is expected to double [1]. It is rare for AD to develop in the 30–50 age group; however, early development of AD can be seen in individuals with mutation in one of the three inherited genes that cause the disease.

In 2016, published research findings suggested that a change in the sense of humor might be an early stage of Alzheimer's. Recent research has indicated that the features of Alzheimer's, such as brain lesions, may already be present in midlife, though the symptoms of the disease do not appear until many years later [2].

The early symptoms of dementia are identified as the reduced ability to take in and remember new information, which can lead to repetitive questioning or conversations, misplacing personal belongings, forgetting events, or getting lost on a familiar route. The most common symptoms observed are as follows:

1. Impairments in reasoning, complex tasking, and exercising judgment, for example, poor understanding of safety risks, inability to manage finances, poor-decision-making ability, and inability to plan complex or sequential activities.

2. Impaired visuospatial abilities due to eyesight problems, inability to recognize faces, common objects, or to find objects in direct view.

3. Impaired speaking, reading, and writing, difficulty in recollecting common words while speaking, hesitations, and spelling and writing errors.

4. Changes in personality and behavior, such as out-of-character, mood change, including agitation, apathy, social withdrawal or a lack of interest, motivation, or initiative, loss of empathy, and compulsive or socially unacceptable behavior.

If the number and severity of symptoms confirm dementia, the following can confirm Alzheimer's. (1) A gradual onset, over the months to years, rather than hours or days. (2) A marked worsening of the individual's normal level of cognition in partial areas [3].

A number of proteins have been found to have a regulatory role in the pathogenesis of AD. Many of these proteins are involved in the morphogenesis, development, and embryogenesis of the organism. Among these, tau and Amyloid Precursor Protein (APP) are the key proteins

in the pathogenesis of sporadic and inherited AD. Thus, developing ways to inhibit the production of these proteins is of increasing research and therapeutic interest. The selective silencing of mutant alleles, moreover, represents an attractive strategy for treating inherited dementias and other dominantly inherited disorders. Here, using tau and APP as model targets is described as an efficient method for producing Small Interfering RNA (siRNA) against any essentially targeted region of a gene. This approach was utilized to develop siRNAs that display optimal allele-specific silencing against a well-characterized tau mutation (V337M) and the most widely studied APP mutation (APPsw). The allele-specific RNA duplexes identified by this method then served as templates for constructing short hairpin RNA (shRNA) plasmids that successfully silenced mutant tau or APP alleles. These plasmids should prove useful in experimental and therapeutic studies of AD. Our results suggest guiding principles for the production of allele-specific siRNA, and the general method described here should facilitate the production of gene-specific siRNAs [4].

2. Causes of AD

AD is caused due to a combination of genetic, lifestyle, and environmental factors that affect the brain over time. Less than 5% of AD is caused by specific genetic changes that virtually guarantee that a person will develop the disease. Although the causes of Alzheimer's are not fully understood yet, its effect on the brain is clear. AD damages and kills the brain cells. A brain affected by AD has fewer cells and much fewer connections among surviving cells than does a healthy brain. As more and more brain cells die, Alzheimer's leads to significant brain shrinkage. When physicians examine an Alzheimer's brain tissue under the microscope, they see two types of abnormalities that are considered to be the hallmarks of the disease:

- **Plaques:** These clumps of a protein called beta-amyloid may damage and destroy brain cells in several ways, including interfering with cell-to-cell communication. Although the ultimate cause of brain-cell death in Alzheimer's is not known, the collection of beta-amyloid on the outside of brain cells is a prime suspect.

- **Tangles:** Brain cells depend on an internal support and transport system to carry nutrients and other essential materials throughout their long extensions. This system requires the normal structure and functioning of a protein called tau. In AD, threads of the tau protein twist into abnormal tangles inside the brain cells, leading to failure of the transport system. This failure is also strongly implicated in the decline and death of brain cells [1,4]. The plaque formation and tangles AD are illustrated diagrammatically in the figure.

3. Diagnosis of Alzheimer's Disease

There is no single test for AD, so physicians look at the signs and symptoms, obtain the medical history, and rule out other conditions before making a diagnosis. They may also check

the individual neurological function, for example, by testing the patient's balance, senses, and reflexes. Other assessments include a blood and urine test, a CT, or a magnetic resonance imaging (MRI) scan of the brain, and screening for depression. Sometimes the symptoms of dementia are related to an inherited disorder such as Huntington's disease, so genetic testing and molecular diagnosis are strongly recommended for the confirmation of Alzheimer's [5].

4. Genes and Genetics of Alzheimer

A gene known as APOE-e4 is associated with higher chances of people above the age of 55 to develop AD [6]. Using this test early could show the symptoms of someone having or developing the disease. However, the test is controversial, and the results are not entirely reliable. In the future, emerging biological tests may make it possible to assess for biomarkers in people who may be at risk of AD.

5. Signaling Mechanism of AD

AD is well studied with its molecular mechanism and detailed signaling topology of the gene network is illustrated. Here, the potential targets were discussed that are essential for the pathogenesis of AD **(Figure 1).** Apart from the general view, a few more genes were also focused to be better therapeutic target.

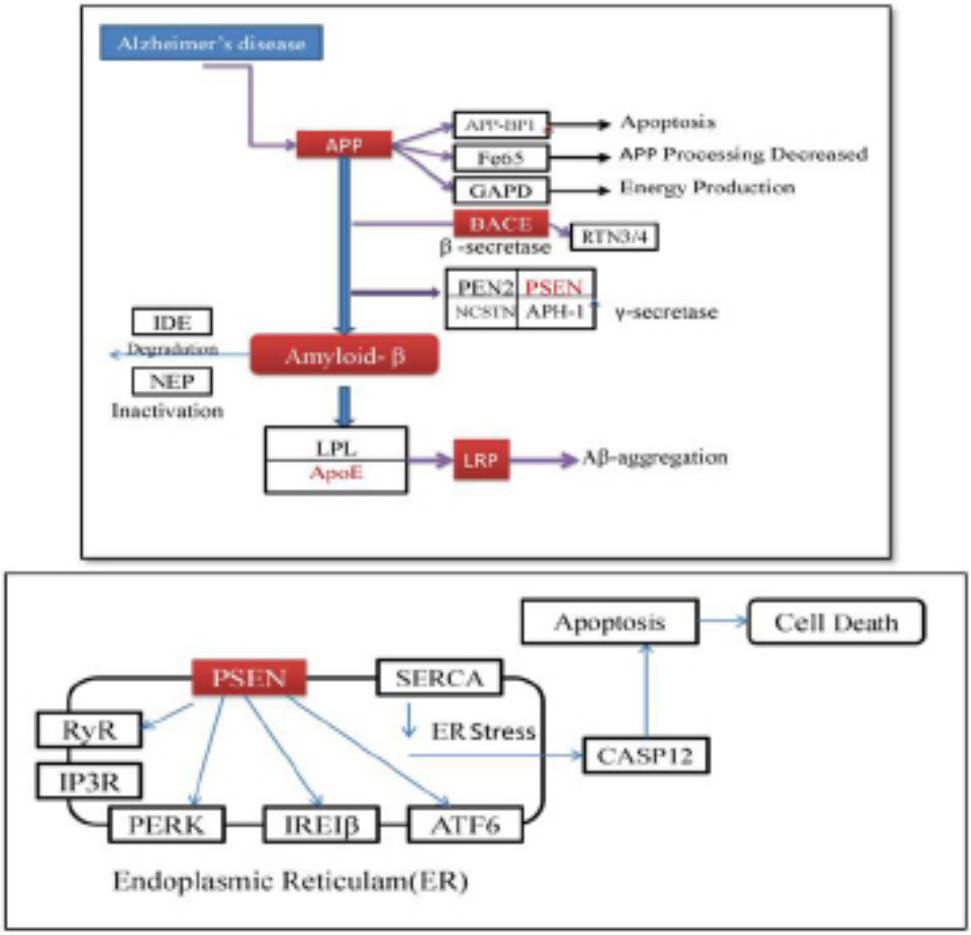

Figure 1: Molecular signaling pathway of the Alzheimer's Disease. The potential gene targets are highlighted in red.

6. Molecular Targets of Alzheimer's

Beta-amyloid (Abeta) protein signaling mechanism is a well-studied pathway for AD. and has a vital role in the pathogenesis of the disease. Data accumulated for well over a decade have implicated the Abeta peptide to be a central player in the pathogenesis of AD **(Figure 1).** Amyloid plaques, composed primarily of Abeta progressively form in the brain of AD patients, and mutations in three genes (APP and presenilin 1 and 2 [PS1 and PS2]) cause early-onset familial AD (FAD) by directly increasing production of the toxic, plaque-promoting Abeta42 peptide. Given the strong association between Abeta and AD, it is likely that therapeutic strategies to lower the levels of Abeta in the brain should prove beneficial for the treatment of AD [7]. One such strategy could involve inhibiting the enzymes that generate Abeta. The Abeta is a product of catabolism of the large type-I membrane protein APP. Two proteases, beta- and gamma-secretase, endoproteolyze APP to liberate the Abeta peptide. Recently, the molecules responsible for these proteolytic activities have been identified. Several lines of evidence suggest that the PS1 and PS2 proteins are gamma-secretase, and the identity of beta-secretase have been shown to be the novel transmembrane aspartic protease, beta-site APP-cleaving enzyme 1 (BACE1; also called Asp2 and memapsin 2). BACE2, a protease homologous to BACE1, was also identified, and together the two enzymes define a new family of transmembrane aspartic proteases. BACE1 exhibits all the functional properties of beta-secretase, and as the key enzyme that initiates the formation of Abeta, it is an attractive drug target for AD. The identification and initial characterization of BACE1 and BACE2, and summaries of recent studies of BACE1 knockout mice have validated BACE1 as the authentic beta-secretase *in vivo* [42]. High throughput screening and various computational studies have been conducted to identify BACE-1 inhibitors [8].

Evidence suggests that the beta-amyloid peptide (Abeta) is central to the pathophysiology of AD. Amyloid plaques, primarily composed of Abeta, progressively develop in the brains of AD patients, and mutations in three genes (APP, PS1, and PS2) cause early onset FAD by directly increasing synthesis of the toxic, plaque-promoting Abeta42 peptide. Given the strong association between Abeta and AD, therapeutic strategies to lower the concentration of Abeta in the brain should prove beneficial for the treatment of AD. One such strategy would involve inhibiting the enzymes that generate Abeta. Abeta is a product of catabolism of the large Type l membrane protein, APP. Two proteases, called beta- and gamma-secretase, mediate the endoproteolysis of APP to liberate the Abeta peptide [9]. For over a decade, the molecular identities of these proteases were unknown. Recently, the gamma-secretase has been tentatively identified as the presenilin proteins, PS1 and PS2, and the identity of the beta-secretase have been shown to be the novel transmembrane aspartic protease, beta-site APP cleaving enzyme 1 (BACE1; also called Asp2 and memapsin2). BACE2, a novel protease homologous to BACE1, was also identified, and together the two enzymes define a new family of transmembrane

aspartic proteases. BACE1 exhibits all the properties of the beta-secretase, and as the key rate-limiting enzyme that initiates the formation of Abeta, BACE1 is an attractive drug target for AD. Here, the identification and initial characterization of BACE1 and BACE2 summarize our current understanding of BACE1 post-translational processing and intracellular trafficking. In addition, recent studies of BACE1 knockout mice and the BACE1 X-ray structure relate implications for BACE1 drug development [10].

6.1. BACE1 Structure and Function in Health and AD

Amyloid plaques, the hallmark neuropathological lesions in the AD brain, are composed of Abeta. Much evidence suggests that Abeta is central to the pathophysiology of AD and is likely to play an early role in this intractable neurodegenerative disorder. Given the strong correlation between Abeta and AD, therapeutic strategies to lower cerebral Abeta levels should prove beneficial for AD treatment. Abeta is derived from APP via cleavage by two proteases, beta- and gamma-secretase [11]. The beta-secretase have been identified as a novel aspartic protease named BACE1 (beta-site APP Cleaving Enzyme (1) that initiates Abeta formation. Importantly, BACE1 appears to be dysregulated in AD. As the rate-limiting enzyme in Abeta generation, BACE1, in principle, is an excellent therapeutic target for strategies to reduce the production of Abeta in AD. While BACE1 knockout (BACE1-/-) mice have been instrumental in validating BACE1 as the authentic beta-secretase *in vivo*, data indicate that complete abolishment of BACE1 may be associated with specific behavioral and physiological alterations [44]. Recently a number of non-APP BACE1 substrates have been identified. It is plausible that failure to process certain BACE1 substrates may underlie some of the reported abnormalities in BACE1-/- mice. Here, they reviewed the basic biology of BACE1, focusing on the regulation, structure, and function of this enzyme. Special attention is given to the putative function of BACE1 during normal conditions and it is discussed in detail the relationship that exists between key risk factors for AD and the pathogenic alterations in BACE1 that are observed in the diseased state [12].

6.1.2. BACE With Small Inhibitory Nucleic Acids

Beta-secretase, a beta-site APP cleaving enzyme (BACE), participates in the secretion of beta-amyloid peptides (Abeta), the major components of the toxic amyloid plaques found in the brains of patients with AD. According to the amyloid hypothesis, accumulation of Abeta is the primary influence driving AD pathogenesis. Lowering of Abeta secretion can be achieved by decreasing BACE activity rather than by down-regulation of the APP substrate protein. Therefore, beta-secretase is a primary target for anti-amyloid therapeutic drug design. Several approaches have been undertaken to find an effective inhibitor of human beta-secretase activity, mostly in the field of peptidomimetic, non-cleavable substrate analogs [41,42]. This review describes strategies targeting BACE mRNA recognition and its down-regulation based

on the antisense action of small inhibitory nucleic acids (siNAs). These include antisense oligonucleotides, catalytic nucleic acids ribozymes and deoxyribozymes as well as small interfering RNAs (siRNAs). While antisense oligonucleotides were first used to identify an aspartyl protease with beta-secretase activity, all the strategies have now demonstrated that siNAs are able to inhibit BACE gene expression in a sequence-specific manner, measured both at the level of its mRNA and at the level of protein. Moreover, knockdown of BACE reduces the intra- and extracellular population of Abeta 40 and Abeta 42 peptides. An anti-amyloid effect of siNAs is observed in a wide spectrum of cell lines as well as in primary cortical neurons. Thus, targeting BACE with small inhibitory nucleic acids may be beneficial for the treatment of AD and for the future drug design [15].

6.2. Amyloid Precursor Protein

APP is a single-pass transmembrane protein expressed at high levels in the brain and metabolized in a rapid and highly complex fashion by a series of sequential proteases, including the intramembranous γ-secretase complex, which also process other key regulatory molecules. The Aβ accumulation in the brains of elderly individuals is unclear, but could relate to changes in APP metabolism or Aβ elimination. Lessons learned from biochemical and genetic studies of APP processing will be crucial for the development of better therapeutic targets for the treatment of AD [14].

6.2.1. Structure And Function of APP

APP is a member of a family of related proteins that include the amyloid precursor-like proteins (APLP1 and APLP2) in mammals and the amyloid precursor protein-like (APPL) in Drosophila. All are single-pass transmembrane proteins with large extracellular domains (Figure 2), and they are all processed in a manner similar to APP. Only APP generates an amyloidogenic fragment owing to sequence divergence at the internal Aβ site [13]. Alternate splicing of the APP transcript generates 8 isoforms, of which 3 are the most common: the 695 amino acid form, expressed predominantly in the CNS and the 751 and 770 amino acid forms. Sequential cleavage of the APP occurs by two pathways. (1) The APP family of proteins has large, biologically active, N-terminal ectodomains as well as a shorter C-terminus that contains a crucial Tyrosine–Glutamic Acid-Asparagine-Proline-Threonine-Tyrosine (YENPTY) protein-sorting domain to which the adaptor proteins X11 and Fe65 bind. The Aβ peptide starts within the ectodomain and continues into the transmembrane region (red). (2) Nonamyloidogenic processing of APP involving α-secretase followed by γ-secretase is shown. (3) Amyloidogenic processing of APP involving BACE1 followed by γ-secretase is shown. Both processes generate soluble ectodomains (sAPPα and sAPPβ) and identical intracellular C-terminal fragments (AICD) [14].

6.2.2. Regulatory role of App in AD

Mutations at codon 717 in exon 17 of the β–APP gene have previously been shown to segregate with early onset AD in some families. The mutation occurs at codons 670 and 671 (APP 770 transcript), the amino terminal of β–amyloid and may be pathogenic because it occurs at or close to the endosomal/lysosomal cleavage site of the molecule. Thus, pathogenic mutations in APP frame the β-amyloid sequence [9].

The APP gene protects against AD and cognitive decline in the elderly without Alzheimer's disease.The coding variants in APP in a set of whole-genome sequence data from 1,795 Icelanders found a coding mutation (A673T) in the APP gene that protects against AD and cognitive decline in the elderly without Alzheimer's disease.The strong protective effect of the A673T substitution against AD provides proof of principle for the hypothesis that reducing the β-cleavage of APP may protect against the disease. Furthermore, as the A673T allele also protects against cognitive decline in the elderly without AD, the two may be mediated through the same or similar mechanisms [10].

The primal role that the amyloid-β (Aβ) peptide has in the development of AD is now almost universally accepted. It is also well recognized that Aβ exists in multiple assembly states, which have different physiological or pathophysiological effects. Although the classical view is that Aβ is deposited extracellularly, emerging evidence from transgenic mice and human patients indicates that this peptide can also accumulate intraneuronally, which may contribute to disease progression [11].

Recent therapeutic investigations of AD have been guided by two seemingly opposed hypotheses: the amyloid cascade theory, which favors the amyloid plaques as the cause of AD; and the cholinergic theory, which favors cholinergic neuron loss as the cause. New investigations indicate that the synthesis and processing of APP are linked to the trophic actions of the nerve growth factor. A pathological cascade in both AD- and Down's syndrome-related memory loss could be triggered by alterations in APP processing or ACh-mediated neuronal function, or both, which in turn trigger the overexpression of amyloid β, synaptic malfunction, and trophic factor loss in target regions. This eventually leads to synaptic and dendritic loss with age [12].

AD is the most common cause of age-related dementia. Pathologically, AD is characterized by the deposition of amyloid-β peptides in the brain, derived from proteolysis of APP by β-site APP cleaving enzyme 1 (BACE1) and γ-secretase. A growing body of evidence implicates cholesterol and cholesterol-rich membrane microdomains in the amyloidogenic processing of APP. Here, we review the recent findings regarding the association of BACE1, γ-secretase, and APP in lipid rafts, and discuss the potential therapeutic strategies for AD that are based on knowledge gleaned from the membrane environment that fosters APP processing.

6.3. Gamma secretase activating protein

The regulator of gamma-secretase activity specifically activates the production of amyloid-beta protein (amyloid-beta protein 40 and amyloid-beta protein 42), without affecting the cleavage of other gamma-secretase targets such as Notch. The gamma-secretase complex is an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (amyloid-beta precursor protein). Specifically it promotes the gamma-cleavage of APP CTF-alpha (also called APP-CTF) by the gamma-secretase complex to generate amyloid-beta, while it reduces the epsilon-cleavage of APP CTF-alpha, leading to a low production of AICD. The gamma-secretase regulator activity is specifically inhibited by imatinib (also known as STI571 or Gleevec), an anticancer drug that selectively decreases amyloid-beta protein production. Imatinib binds PION/GSAP and acts by preventing PION/GSAP interaction with the gamma-secretase substrate. Its role as an activator of amyloid-beta protein production makes it a promising therapeutic target for the treatment of AD [17]. Activation of the γ-secretase complex is required for the final formation of Aβ peptides, and decreasing Aβ production by blocking this complex as a disease modifying approach for the treatment of AD has received intense investigation [18]. However, γ-secretase is known to process multiple substrates in addition to APP, most notably Notch, and this fact has severely limited the clinical development of inhibitors directly and irreversibly targeting this enzyme. The recent discovery of a γ-secretase activating protein (GSAP) which interacts with this protease to facilitate Aβ formation without affecting Notch has established it as a relevant target for a viable and safer anti-Aβ therapy. GSAP is increased in postmortem brain tissues of AD patients, and its pharmacological or genetic inhibition results in an amelioration of the AD-like amyloidotic phenotype in transgenic mouse models of the disease [19,57].

6.3.1. Role of GSAP in AD

Gamma-secretase is a large enzyme complex comprising presenilin, nicastrin, presenilin enhancer 2, and anterior pharynx-defective that mediates the intramembrane proteolysis of a large number of proteins including APP and Notch. Recently, a novel GSAP was identified that interacts with Gamma-secretase and the C-terminal fragment of APP to selectively increase amyloid-beta production. In this study, the role of endogenous and exogenous GSAP in the regulation of Gamma-secretase activity and amyloid-Beta production *in vitro* is further characterized. Knockdown of GSAP expression in N2a cells decreases amyloid-beta levels. In contrast, overexpression of GSAP in HEK cells expressing APP or in N2a cells had no effect on amyloid-beta generation. Likewise, purified recombinant GSAP had no effect on amyloid-β generation in two distinct *in vitro* Gamma-secretase assays. In subsequent cellular studies with imatinib, a kinase inhibitor that reportedly prevents the interaction of GSAP with the C-terminal fragment of APP, a concentration-dependent decrease in amyloid-beta levels was observed. However, no interaction between GSAP and the C-terminal fragment of APP was

evident in co-immunoprecipitation studies. In addition, sub-chronic administration of imatinib on rats had no effect on the brain amyloid-beta levels. In summary, these findings suggest that the roles of GSAP and imatinib in the regulation of Gamma secretase activity and amyloid-Beta generation are uncertain [20].

6.4. Tau protein

Tau is a major MAP in the brain, but in this regard it is not more or less interesting than other MAPs that have been discovered and classified over the years (MAP1, MAP2, MAP4, etc.). The major interest in tau stems from its aggregation in AD and other tauopathies. There has been a debate on whether tau is causal to the disease or just a byproduct of some disease process. In the case of AD the case is still open, and changes in tau are mostly viewed as a consequence of Aβ pathology. However, the discovery of mutations in the tau gene causing frontotemporal dementias has confirmed a causative role of tau in neurodegeneration, as well as the identification of Tau as one of the risk factors in PSP, PD, and others. Even in the context of AD, the active contribution of Tau was highlighted by animal models, suggesting that tau is required for the induction of Aβ-induced toxicity. In fact, an increased Tau level alone suffices as a risk factor, as demonstrated for the H1c haplotype. This provides a rationale for the quest for tau-lowering drugs [21].

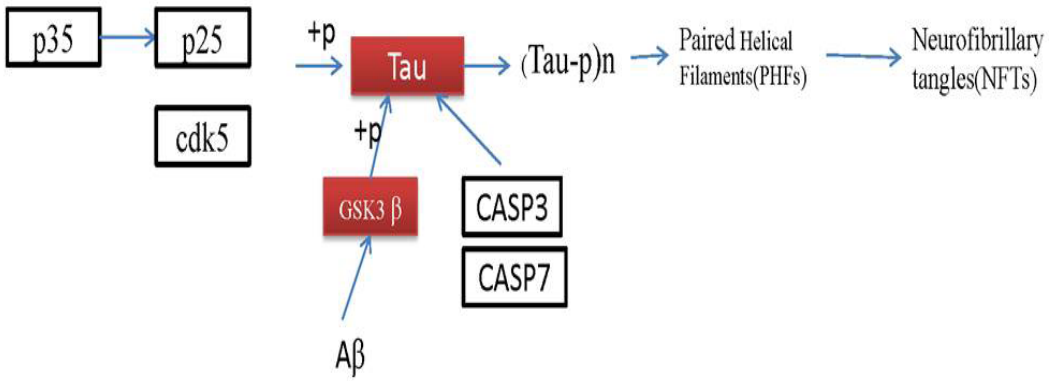

Figure 2: Signaling mechanism of tau protein with characteristic pathological features.

6.4.1. Tau protein isolation and localization

The tau (tubulin-associated unit) protein was isolated from porcine brain extracts as a heat-stable, highly soluble protein essential for microtubule (MT) assembly. Following the initial discovery of tau, two studies reported the process of tau purification and its physical and chemical properties, including the ability of tau to become phosphorylated **(Figure 2)**. In 1983, it was discovered that tau could be phosphorylated at multiple sites by various protein kinases, including cyclic-AMP-dependent protein kinases and casein kinase type-1. Further studies showed that tau is a phosphoprotein and that phosphorylation negatively regulates its ability to stimulate MT assembly. An immunohistochemical study that compared the localization of

tau using the tau-1 antibody (that recognizes all isoforms of tau) with that of microtubule-associated protein 2 (MAP2) and tubulin in human postmortem brain tissue demonstrated that tau protein was primarily localized to axons. Using the same tau-1 monoclonal antibody and electron microscopy with colloidal gold-labeled secondary antibodies, tau were also found in very low amounts in astrocytes and oligodendrocytes, and this was confirmed by tau mRNA expression analysis in the mouse brain [22].

6.4.2. Regulatory role of tau protein

Functions of the tau protein is most abundantly expressed in axons of central nervous system neurons, but can also be found in the somatodendritic compartment of neurons, oligodendrocytes, and non-neural tissues. Probably the most important role of the tau protein is to promote the assembly and stability of MT, although this function is complemented by other MAP (especially by MAP1B), as tau knockout mice are viable, fertile, and relatively normal, with no signs of neurodegeneration. Also, knockdown of tau with small interfering RNA does not kill primary neurons in culture or prevent axon formation. Additionally, MAP1B is probably more important for MT stability than tau itself, because knockout of MAP1B results in abnormal brain development and early death, and concurrent knockout of both MAP1B and *MAPT* worsens the phenotype. The most common post-translational modifications of tau proteins are phosphorylation and O-glycosylation. Phosphorylation changes the shape of tau molecule and regulates its biological activity. Most of the phosphorylation sites are on Ser-Pro and Thr-Pro motives, but a number of sites on other residues have also been reported. The majority of tau-based therapeutic strategies against neurodegeneration have focused on modulating tau phosphorylation, given that tau species present within NFT are hyperphosphorylated. O-glycosylation is characterized by the addition of an *O*-linked *N*-acetylglucosamine(*O*-GlcNAc) on Ser or Thr residues in the vicinity of Pro residues. It is presumed that glycosylation may have a role in subcellular localization and degradation of tau proteins. The recent discovery that tau is also modified by acetylation requires additional research to provide greater insight into the physiological and pathological consequences of tau acetylation.

Tau protein can be divided into two main functional domains: the basic MT binding domain (toward the C-terminus) and the acidic projection domain (toward the N-terminus). The MT binding domain regulates the rate of MT polymerization through highly conserved repetitive domains R1–R4 encoded by exons 9–12. Adult tau isoforms with 4R (R1–R4) are about 40-fold more efficient at promoting MT assembly than the fetal isoform that lacks exon 10 and thus has only 3R. The absence of expression of the R1–R2 inter-repeat region during fetal development allows for the cytoskeletal plasticity required of growing immature neurons and their elongating axons. Apart from binding to MT, the repeat domains of tau also bind to tubulin deacetylase, histone deacetylase 6 (HDAC6), and apolipoprotein E (apoE, more with

the ε3 than the ε4 isovariant) [24].

6.4.3. Role of tau protein in AD

The sequences of isoforms of human tau protein differ from previously reported forms by insertions of 29 or 58 amino acids in the amino-terminal region. Complementary DNA cloning shows that the insertions occur in combination with both three and four tandem repeats. RNAase protection assays indicate that transcripts encoding isoforms with the insertions are expressed in an adult-specific manner. Transcripts encoding four tandem repeats are also expressed in an adult-specific manner, whereas mRNAs encoding three tandem repeats are expressed throughout life, including in fetal brain. The levels of transcripts encoding the 29 or 58 amino acid inserts were not significantly changed in the cerebral cortex of patients with AD. Antisera raised against synthetic peptides corresponding to these different human tau isoforms demonstrate that multiple tau protein isoforms are incorporated into the neurofibrillary tangles of AD [22].

Glycogen synthase kinase-3 (GSK-3) reduced the mobility of human tau on SDS-PAGE, prevented binding of the monoclonal antibody (mAb), Tau.1, and induced binding of the mAb 8D8. Recombinant tau phosphorylated by GSK-3 aligned on SDS-PAGE with the abnormally phosphorylated tau (PHF-tau) associated with the paired helical filaments in AD brain. Phosphorylated serine^{396} (numbering of the large human brain tau isoform) was identified as a binding site on tau for mAb 8D8. The localization of GSK-3 within granular structures in pyramidal cells indicates that GSK-3α and GSK-3β may have a role in the production of PHF-tau in AD [24]. Paired helical filaments (PHFs) are a characteristic pathological feature of AD; their principal component is the microtubule-associated protein tau. The tau in PHFs (PHF-tau) is hyperphosphorylated, but the cellular mechanisms responsible for this hyperphosphorylation have yet to be elucidated. A number of kinases, including mitogen-activated protein (MAP) kinase, glycogen synthase kinase (GSK)-3*α*, GSK-3*β* and cyclin-dependent kinase-5, phosphorylate recombinant tau *in vitro* so that it resembles PHF-tau as judged by its reactivity with a panel of antibodies capable of discriminating between normal tau and PHF-tau, and by a reduced electrophoretic mobility that is characteristic of PHF-tau. To determine whether MAP kinase, GSK-3*α* and GSK-3*β* can also induce AD -like phosphorylation of tau in mammalian cells, the phosphorylation status of tau in primary neuronal cultures and transfected COS cells following changes in the activities of MAP kinase and GSK-3 was studied. Activating MAP kinase in cultures of primary neurons or transfected COS cells expressing tau isoforms did not increase the level of phosphorylation for any PHF-tau epitope investigated. However, elevating GSK-3 activity in the COS cells by co-transfection with GSK-3α or GSK-3*β* decreased the electrophoretic mobility of tau so that it resembled that of PHF-tau, and induced reactivity with eight PHF-tau-selective monoclonal antibodies.The data indicate that GSK-3*α* and/or GSK-3*β*, but not MAP kinase, are good candidates for generating PHF-type phosphorylation

of tau in AD. The involvement of other kinases in the generation of PHFs cannot, however, be eliminated. Our results suggest that aberrant regulation of GSK-3 may be a pathogenic mechanism in AD [25].

6.5 APOE Protein

AD affects over 30 million people worldwide and one in nine people above 65 years of age [26]. AD is characterized clinically by brain shrinkage accompanied by progressive memory loss and cognitive decline as well as personality changes later in the disease course. Pathologically, AD is characterized by the progressive accumulation of neuritic plaques of amyloid-beta (Ab) followed by neurofibrillary tangles of hyperphosphorylated tau. Overt clinical symptoms typically do not appear until the underlying pathology is well developed [27]; however, functional imaging studies suggest that changes in synaptic function occur several years before outward signs of the disease are apparent [26]. Moreover, rising Ab levels may in part be responsible for the subtle, but also progressive, reduction in cognitive ability that occurs during normal aging, and patients with "subjective cognitive decline" (i.e., patients who perform normally on standardized memory tests, but nevertheless report subjective memory impairment) have generally higher levels of Ab deposition on positron emission tomography (PET) [27].

Over two decades ago, ApoE4 was identified as a major genetic risk factor for late-onset AD (i.e., after 60 years of age) [26]. Possession of one copy of ApoE4 triples the risk of developing AD, while individuals with two copies have a 90% lifetime risk of developing the disease. The allele frequency ApoE4 is 15%–20%, with some variation in incidence between populations. Conversely, ApoE2 is considered to be protective against AD, while ApoE3 is considered risk neutral because it is by far the most common of the three isoforms and, thus, is considered the standard for the general population [26].

Since its identification as an important risk factor, great strides have been made in understanding the role that ApoE4 plays in synapse function and AD. One important role of ApoE is the clearance of Ab, with ApoE4 hindering Ab clearance significantly over ApoE3 and ApoE2 and thus directly increasing amyloid pathology. Additional roles for ApoE4 have been indicated by noninvasive imaging studies, which have shown that older individuals who are ApoE4 carriers have structural and functional alterations in AD-affected areas in the absence of cognitive dysfunction. Moreover, some of these changes are present much earlier in life, indicating a role for ApoE in neuronal function before amyloid deposition. There is an enormous literature exploring the interaction between ApoE4 and Ab, which has been reviewed in-depth recently; therefore, this review focuses on the roles of ApoE and its receptors at the synapse.

6.5.1 Role of APOE in AD

As the population ages, neurodegenerative diseases such as AD are becoming a significant burden on patients, their families, and healthcare systems. Neurodegenerative processes may start up to 15 years before outward signs and symptoms of AD, as evidenced by data from AD patients and mouse models. A major genetic risk factor for late-onset AD is the e4 isoform of apolipoprotein E (ApoE4), which is present in almost 20% of the population. The contribution of ApoE receptor signaling to the function of each component of the tripartite synapse, the axon terminal postsynaptic dendritic spine, and the astrocyte and examine how these systems fail in the contexts of ApoE4 and AD [27].

Among other metabolic functions, the apolipoprotein E (APOE) plays a crucial role in neuroinflammation. Aiming at assessing whether *APOE* ε4 modulates levels of glial cerebrospinal fluid (CSF) biomarkers and their structural cerebral correlates along the continuum of AD, brain MRI scans were acquired in 110 participants (49 control; 19 preclinical; 27 mild cognitive impairment [MCI] due to AD; 15 mild AD dementia) and CSF concentrations of YKL-40 and sTREM2 were determined. Differences in CSF biomarker concentrations and interactions in their association with gray-matter volume, according to *APOE* ε4 status were sought after. Preclinical and MCI carriers showed higher YKL-40 levels. There was a significant interaction in the association between YKL-40 levels and gray-matter volume according to ε4 status. No similar effects could be detected at sTREM2 levels, an indication of increased astroglial activation in APOE ε4 carriers while both groups displayed similar levels of CSF AD core biomarkers.

APOE4, identified in 1993, is the greatest genetic risk factor for sporadic AD, increasing the risk by up to 15-fold compared with *APOE3*, with *APOE2* decreasing the AD risk. However, the functional effects of *APOE4* on AD pathology remain unclear and, in some cases, controversial. *In vivo* progress to understand how the human (h)-*APOE* genotypes affect AD pathology has been limited by the lack of a tractable familial AD-transgenic (FAD-Tg) mouse model expressing h-*APOE* rather than mouse (m)-*APOE.* The disparity between m- and h-apoE is relevant for virtually every AD-relevant pathway, including amyloid-β (Aβ) deposition and clearance, neuroinflammation, tau pathology, neural plasticity, and cerebrovascular deficits. EFAD mice were designed as temporally useful preclinical FAD-Tg-mouse models expressing the h-*APOE* genotypes for identifying mechanisms underlying *APOE*-modulated symptoms of AD pathology. From their first description in 2012, EFAD mice have enabled critical basic and therapeutic research. Here, we review insights gleaned from the EFAD mice and summarize the future directions [28]. In 2012, we studied computationally to identify the potential ApoE4 inhibitor from plant compounds. Rigid docking study was performed for 18 plant compounds and 11 cholinesterase inhibitors. Based on the docking score, binding energy and number of hydrogen bonding curcumin possess the best scoring function. For further validation, induced

fit docking was performed which also showed that curcumin binds to the same binding pocket of ApoE4 protein. Biological activity prediction reveals that curcumin has a potential therapeutic activity against AD. Pharmacokinetic properties of this compound are under the acceptable range. From the results obtained, we concluded that the plant compound curcumin could be a potential inhibitor of ApoE4 and it can control the AD [28].

6.6. Presenilin (PSEN) protein

The first clue to the role of presenilins in APP processing came from observations that AD-causing mutations in *PSEN1* and *PSEN2* (more than 150 different mutations in these genes have been identified) affect the generation of Aβ peptides, changing the relative amount of Aβ42 peptide (Aβ containing 42 amino acid residues) versus the shorter Aβ40 (the more abundantly generated peptide, containing 40 amino acid residues; [51]. This was shown in fibroblasts derived from patients [52], by overexpressing the mutant presenilins in cell lines [53,55] and by experiments in living mice, either overexpressing the mutant presenilin in the brain using various promoters [53,54,55] or by knocking in mutations in the endogenous mouse presenilin gene [56].

The function of presenilin in the γ-secretase proteolytic activity became apparent when neurons were derived from *PSEN1* knockout mice and were used to show that PSEN1 was critically involved in the generation of all Aβ peptides [57]. This experiment established presenilin as an important AD drug target. The central role of presenilin in the γ-secretase processing of Notch was established a year later in mouse and Drosophila [57,58]. Furthermore, because a γ-secretase inhibitor was shown to block not only APP processing, but also Notch cleavage, it was suggested that a presenilin-dependent protease was responsible for both cleavages, and that blocking this enzyme would cause major side effects in patients. Notch is indeed not only involved in embryogenesis and development, but also in differentiation of immune cells, the goblet cells in the intestine, and others [58].

At the same time, other studies suggest that presenilin was actually the catalytic subunit of γ-secretase. Site-directed mutagenesis of two aspartyl residues embedded in the TMDs VI and VII of PSEN1 resulted in a dominant-negative effect on γ-secretase activity, suggesting that presenilin was a protease, specifically of the aspartyl type. These mutations did not affect the expression or the incorporation of presenilin into the γ-secretase complex, and are in a conserved region of the presenilin proteins [59]. They are found in a family of related intramembrane-cleaving proteases, the signal peptide peptidases (SPP) [60]. Finally, transition-state analog (i.e., active site-directed) γ-secretase inhibitors were shown to directly bind to the presenilin subunit of the γ-secretase complex, providing convincing evidence that presenilin is indeed a protease.

In mammals, two homologous proteins exist, PSEN1 and PSEN2. They are both synthesized as precursor proteins of 50 kDa with nine TMDs, and are cleaved into a 30 kDa amino-terminal fragment (NTF) and a 20 kDa carboxy-terminal fragment (CTF) during maturation, probably by autocatalysis [35].

6.6.1. Role of PSEN in AD

Genetic causes of AD include mutations in the *APP*, *presenilin1* (PS1), and *presenilin2* (P52) genes. The mutant *APP k670N,M67M* transgenic line, Tg2576, shows markedly elevated amyloid β-protein (AP) levels at an early age and, by 9–12 months, develops extracellular AD-type Ap deposits in the cortex and hippocampus. Mutant *PS1* transgenic mice do not show abnormal pathology, but display subtly elevated levels of the highly amyloidogenic 42- or 43-amino acid peptide Aβ342(43). The doubly transgenic progeny from a cross between line Tg2576 and a mutant *PS1 M46L* transgenic line develop large numbers of fibrillar Aβ deposits in the cerebral cortex and hippocampus far earlier than their singly transgenic Tg2576 littermates. In the period preceding overt Aβ deposition, the doubly transgenic mice show a selective 41% increase in Aβ42(43) in their brains. Thus, the development of AD-like pathology is substantially enhanced when a P51 mutation, which causes a modest increase in Aβ42(43), is introduced into Tg2576-derived mice. Remarkably, both doubly and singly transgenic mice showed reduced spontaneous alternation performance in a "Y" maze before the substantial Aβ deposition was apparent. This suggests that some aspects of the behavioral phenotype in these mice may be related to an event that precedes plaque formation [36].

Presenilin-1 and -2 (PS1 and PS2) mutations, the major cause of FAD, have been causally implicated in the pathogenesis of neuronal cell death through a perturbation of cellular Ca^{2+} homeostasis. They have recently shown that, at variance with previous suggestions obtained in cells expressing other FAD-linked PS mutations, PS2-M239I and PS2-T122R cause a reduction and not an increase in cytosolic Ca^{2+} rises induced by Ca^{2+} release from the stores. In this study, we have used different cell models: human fibroblasts from controls and FAD patients, cell lines (SH-SY5Y, HeLa, HEK293, MEFs) and rat primary neurons expressing a number of PS mutations, e.g. P117L, M146L, L286V, and A246E in PS1 and M239I, T122R, and N141I in PS2. The effects of FAD-linked PS mutations on cytosolic Ca^{2+} changes have been monitored either by using fura-2 or recombinant cytosolic aequorin as the probe. Independently of the cell model or the employed probe, the cytosolic Ca^{2+} increases, caused by agonist stimulation or full store depletion by drug treatment, were reduced or unchanged in cells expressing the PS mutations. Using aequorins, targeted to the endoplasmic reticulum or the Golgi apparatus, shows that FAD-linked PS mutants lower the Ca^{2+} content of intracellular stores. The phenomenon was most prominent in cells expressing PS2 mutants, and was also observed in cells expressing the nonpathogenic, "loss-of-function" PS2-D366A mutation, while confirming the capability of presenilins to modify Ca^{2+} homeostasis suggests a reevaluation of the "Ca^{2+}overload"

hypothesis in AD and a new working hypothesis is presented [37].

The BRCA1 protein, one of the major players responsible for DNA damage response has recently been linked to AD. Using primary fibroblasts and neurons reprogrammed from induced pluripotent stem cells (iPSC) derived from FAD patients the role of the BRCA1 protein underlying molecular neurodegeneration. By whole-transcriptome approach, there was wide range of disturbances in cell cycle and DNA damage response in FAD fibroblasts. This was manifested by the significantly increased content of BRCA1 phosphorylated on Ser1524 and the abnormal ubiquitination and subcellular distribution of presenilin 1 (PS1). Accordingly, the iPSC-derived FAD neurons showed increased content of BRCA1(Ser1524) co-localized with degraded PS1, accompanied by an enhanced immunostaining pattern of amyloid-β. Finally, overactivation of BRCA1 was followed by an increased content of Cdc25C phosphorylated on Ser216, likely triggering cell cycle re-entry in FAD neurons. This study suggests that overactivated BRCA1 could both influence PS1 turnover leading to amyloid-β pathology and promote cell cycle re-entry-driven cell death of postmitotic neurons in AD [38].

6.7. LDL Receptor-Related Protein

The LDL receptor-related protein (LRP) is larger than but structurally similar to other members of the LDL receptor gene family, an ancient family of endocytic receptors.Whereas the LDL receptor, the founding member of this family, appears to act solely in lipoprotein metabolism, the LRP and other members of this family appear to have other distinct functions. The diverse biological roles of the LRP, include functions in lipid metabolism, and also in the homeostasis of proteinases and proteinase inhibitors, cellular entry of viruses and toxins, activation of lysosomal enzymes, cellular signal transduction, and neurotransmission.

6.7.1. Structural organization of LRP

LRP, like all members of the LDL receptor gene family, consists of five common structural units: (1) ligand-binding (complement) type cysteine-rich repeats, (2) epidermal growth factor (EGF) receptor–like cysteine-rich repeats, (3) YWTD domains, (4) a single membrane-spanning segment, and (5) a cytoplasmic tail that harbors between one and three NPxY motifs (Figure 3). Ligand-binding-type repeats in LRP occur in clusters containing between 2 and 11 individual repeats. Most of the known ligands for LRP, for which the binding sites have been mapped, interact with these ligand-binding-type domains. These are followed by EGF precursor homology domains, which consist of the two EGF repeats, six YWTD repeats that are arranged in a propeller-like structure, and another EGF repeat. Six EGF repeats precede the single membrane-spanning segment. NPxY motifs that serve as docking sites for the endocytosis machinery and forcytoplasmic adaptor and scaffolding proteins involved in signaling events [39].

Binding of LRP ligands to the different clusters of ligand-binding repeats. Cysteine-rich ligand-binding repeats (red ovals) in LRP are arranged in four clusters containing 2, 8, 10, and 11 repeats, respectively. Each cluster is followed by 1–4 EGF homology domains (blue), which consist of cysteine-rich EGF repeats (blue circles) and YWTD domains (wavy line). NPxY motifs in the cytoplasmic tail are indicated by the asterisks. No ligand interactions have been mapped to cluster I. Clusters II and IV bind most of the currently mapped known ligands of LRP binding of α_2M to clusters II and IV found by surface plasmon resonance, although cells transfected with minireceptors containing these clusters do not bind and internalize α_2M. ApoE was found to bind to clusters II, III, and IV by ligand blotting. LPL, lipoprotein lipase [39].

6.7.2. LRP Potential Role in AD

A number of findings suggest that LRP contributes to the pathobiology of AD. LRP serves as a receptor for APP, apoE, and α_2M, all of which have been genetically linked to AD. Furthermore, the levels of LRP decrease substantially with age, the major risk factor for nonfamilial AD. The contribution of LRP to AD is complex, and studies demonstrate that LRP has the capacity to influence both the production and the clearance of Aβ. The association of LRP with forms of APP that contain a Kunitz-type proteinase inhibitor (KPI) domain alters APP processing, leading to increased Aβ production. At the same time, the Aβ peptide binds avidly to LRP ligands, such as α2M and apoE, and LRP-mediated clearance of these ligands complexed to Aβ contributes to a reduction in Aβ levels. Interestingly, a silent polymorphism in exon 3 of the LRP gene (C776T) is associated with an altered risk for late-onset AD and significantly lower levels of LRP in the brain have been reported in AD patients with the C/C genotype compared with patients with the C/T or T/T genotype. Decreased LRP expression at clearance sites (perhaps at neurons or at sites along the capillary membranes) could lead to decreased α2M* and/or apoE-promoted Aβ catabolism, resulting in increased Aβ deposition.

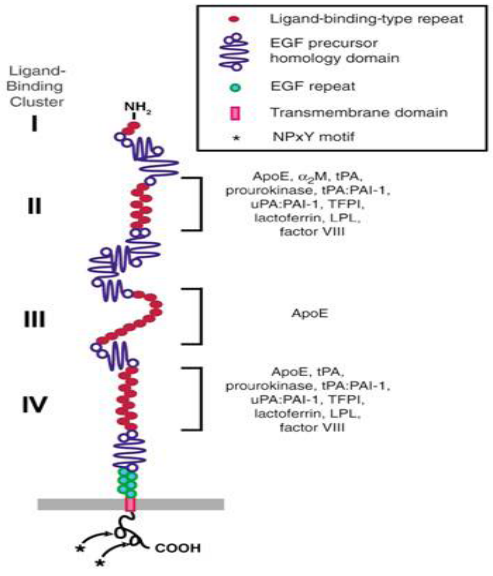

Figure 3: Structural organization of LRP. Binding of LRP ligands to the different clusters of ligand-binding repeats [39].

At the same time, increased expression of LRP in activated glia in the AD brain is well documented and could lead to increased Aβ production by these cells, also leading to increased Aβ deposition. Finally, the signaling roles of LRP in response to ligand binding may be important for normal synaptic plasticity, and loss of LRP function or levels may impair these processes and lead to neuronal degeneration[39].

6.7.3. Role of hydrophobic patches in LRP6

LRP6 protein is found in the senile plaques of AD patients. Inducing the disassociation/ inhibition of the LRP6–DKK1 complex is a vital mechanism for the treatment of AD. LRP6 is an important receptor in the Wnt/β catenin canonical pathway, where DKK1 binds in the normal state as a natural antagonist, while in the extreme level of disease, DKK1 unbinds from the LRP6, giving privilege for Wnt signaling pathway to activate the TCF/LEF genes. In 2016, we published a research article on the role of hydrophobic pathches in LRP6. Inorder to check the effectiveness of the ligands, docking was performed on the active site of DKK1 and on the hydrophobic patch of LRP6 where DKK1 binds. Ligands interacting on the active site of DKK1 and residues interacting on the hydrophobic patch of LRP6 were confirmed based on good Glide score and Glide energy. Ligands can bind to the active site of the hydrophobic patch on LRP6 with the same efficacy as DKK1 binds to LRP6 as a natural antagonist. This study accomplished its goal of targeting potent inhibitors against LRP6 by molecular modeling techniques such as high throughput virtual screening and molecular dynamics simulations [61].

6.8. Pkcδ as a target for AD

The β-amyloid protein (Aβ) plays a central role in the pathogenesis of AD. Aβ is generated from the sequential cleavage of APP by β-site APP-cleaving enzyme 1 (BACE1) and the γ-secretase complex. Although activation of some protein kinase C (PKC) isoforms such as PKCα and ε has been shown to regulate nonamyloidogenic pathways and Aβ degradation, it is unclear whether other PKC isoforms are involved in APP processing/AD pathogenesis. In this study, we report that increased PKCδ levels correlate with BACE1 expression in the AD brain. PKCδ knockdown reduces BACE1 expression, BACE1-mediated APP processing, and Aβ production. Conversely, overexpression of PKCδ increases BACE1 expression and Aβ generation. Importantly, inhibition of PKCδ by rottlerin markedly reduces BACE1 expression, Aβ levels, and neuritic plaque formation and rescues cognitive deficits in APP Swedish mutations K594N/M595L/presenilin-1 with an exon 9 deletion-transgenic AD mouse model. The PKCδ plays an important role in aggravating AD pathogenesis, and PKCδ may be a potential target in AD therapeutics [41].

7. Molecular Pathogenesis of AD

Accumulation of neurotoxic βamyloid (Aβ) is a major hallmark of AD. Formation of Aβ is catalyzed by γsecretase, a protease with numerous substrates 2,3. Little is known about the molecular mechanisms that confer substrate specificity on this potentially promiscuous enzyme. Knowledge of the mechanisms underlying its selectivity is critical for the development of clinically effective γ-secretase inhibitors that can reduce Aβ formation without impairing cleavage of other γ-secretase substrates, especially Notch, which is essential for normal biological functions 3,4. Here we report the discovery of a novel γ-secretase activating protein (gSAP), which dramatically and selectively increases Aβ production through a mechanism involving its interactions with both γsecretase and its substrate, the APP C-terminal fragment (APP-CTF). The gSAP does not interact with Notch nor does it affect its cleavage. Recombinant gSAP stimulates Aβ production *in vitro*. Reducing gSAP levels in cell lines decreases Aβ levels. Knockdown of gSAP in a mouse model of AD reduces the levels of Aβ and plaque development. gSAP represents a new type of γ-secretase regulator that directs enzyme specificity by interacting with a specific substrate, demonstrating that imatinib, as an anticancer drug previously found to inhibit Aβ formation without affecting Notch cleavage5, achieves its Aβ-lowering effect by preventing gSAP interaction with the γ-secretase substrate, APP-CTF. Thus, gSAP can serve as an Aβ-lowering therapeutic target without affecting other key functions of γ-secretase [21].

The γ-Secretase-mediated cleavage of APP results in the production of AD -related amyloid-β (Aβ) peptides. The Aβ42 peptide in particular plays a pivotal role in AD pathogenesis and represents a major drug target. Several γ-secretase modulators (GSMs), such as the nonsteroidal anti-inflammatory drugs (*R*)-flurbiprofen and sulindac sulfide, have been suggested to modulate the Alzheimer-related Aβ production by targeting the APP. The novelty of GSMs is that they are selective for Aβ modulation and do not impair processing of Notch, EphB2, or EphA4. The GSMs modulate Aβ both in cell and cell-free systems as well as lower amyloidogenic Aβ42 levels in the mouse brain. Both radioligand binding and cellular cross-competition experiments reveal a competitive relationship between the AstraZeneca (AZ) GSMs and the established second-generation GSM, E2012, but a noncompetitive interaction between AZ GSMs and the first-generation GSMs (*R*)-flurbiprofen and sulindac sulfide. The binding of a ^{3}H-labeled AZ GSM analog does not co-localize with APP but overlaps anatomically with a γ-secretase targeting inhibitor in rodent brains. Combined, these data provide compelling evidence of a growing class of *in vivo* active GSMs, which are selective for Aβ modulation and have a different mechanism of action compared with the original class of GSMs described [22].

8. Computational Studies in AD

Several targets/signaling mechanisms were reported in the pathogenesis of AD. Among these signaling mechanisms, they are not together possible to test in *in vitro, in vivo* studies. Therefore, these targets can be targeted and validated by computational studies. Through a series of computational studies, we were able to find several inhibitors, theoretically checked against all the possible targets for AD. Further, the screened compounds can be experimentally validated for clinical applications. Such kind of model proteins studied through computational studies were discussed.

9. Recent Developments in the Drug Treatment of AD

Several pharmacological approaches to enhance the cholinergic function have been developed for symptomatic or palliative therapy of AD. Although these strategies have resulted in modest cognitive and behavioral improvements in patients with AD, they do not address the underlying progression of the disease. New strategies will be required to slow, stop, or reverse the effects of neuro-degeneration in AD. A number of potential therapies are currently under investigation, including estrogen replacement, anti-inflammatory agents, free radical scavengers and antioxidants, and monoamine oxidase-B (MAO-B) inhibitors. The evidence for a protective effect of estrogens or nonsteroidal anti-inflammatory drugs (NSAIDs) is controversial, and is largely based on retrospective studies. More controlled prospective studies are needed to definitively demonstrate the benefits of long-term estrogen or NS AID use in the prevention of AD. Free radical scavengers/antioxidants such as idebenone, and selective prevention MAO-B inhibitors such as lazabemide are well tolerated, but require additional studies to demonstrate their preventative effects. In addition, other approaches, such as anti-amyloid treatments that affect beta-amylase secretion, aggregation, and toxicity, appear promising; treatments that hinder neurofibrillary tangle construction and nerve growth factor (NGF) induction are in the very early stages of development [13–15].

Numerous epidemiological studies have shown a significantly higher risk for development of AD in patients affected by type 2 diabetes (T2D), but the molecular mechanism responsible for this association is presently unknown. Both diseases are considered protein misfolding disorders associated with the accumulation of protein aggregates; amyloid-beta (Aβ) and tau in the brain during AD, and islet amyloid polypeptide (IAPP) in pancreatic islets in T2D. The formation and accumulation of these proteins follow a seeding-nucleation model, where a misfolded aggregate or "seed" promotes the rapid misfolding and aggregation of the native protein. Our underlying hypothesis is that misfolded IAPP produced in T2D potentiates AD pathology by cross-seeding Aβ, providing a molecular explanation for the link between these diseases. Here, we examined how misfolded IAPP affects Aβ aggregation and AD pathology *in vitro* and *in vivo*. We observed that addition of IAPP seeds accelerates Aβ aggregation *in vitro*

in a seeding-like manner and the resulting fibrils are composed of both peptides. Transgenic animals expressing both human proteins exhibited exacerbated AD-like pathology compared with AD transgenic mice or AD transgenic animals with type 1 diabetes (T1D). Remarkably, IAPP co-localized with amyloid plaques in brain parenchymal deposits, suggesting that these peptides may directly interact and aggravate the disease. Furthermore, inoculation of pancreatic IAPP aggregates into the brains of AD transgenic mice resulted in more severe AD pathology and significantly greater memory impairments than untreated animals. These data provide a proof-of-concept for a new disease mechanism involving the interaction of misfolded proteins through cross-seeding events which may contribute to accelerate or exacerbate disease pathogenesis. Our findings could shed light on understanding the linkage between T2D and AD, two of the most prevalent protein misfolding disorders [28].

Critical review studies that used electroencephalography (EEG) or event-related potential (ERP) indices as a biomarker of AD are discussed. In the first part studies that relied on visual inspection of EEG traces and spectral characteristics of EEG. Second, the survey analysis methods motivated by dynamical systems theory (DST) as well as more recent network connectivity approaches. The third part contains studies of sleep. Next, compared the utility of early and late ERP components in dementia research. The section on mismatch negativity (MMN) studies summarize their results and limitations and outline the emerging field of computational neurology. In the following overview are the use of EEG in the differential diagnosis of the most common neurocognitive disorders. Finally, the summary of the state of the field and the conclusion that several promising EEG/ERP indices of synaptic neurotransmission are worth considering as potential biomarkers. Furthermore, some practical issues are highlighted with discussion of future challenges as well [29].

Therapeutic treatments for AD include the cholinesterase inhibitors donepezil, galantamine, and rivastigmine. A review of the evidence by searching MEDLINE, Embase, The Cochrane Library and the International Pharmaceutical Abstracts from 1980 through 2007 (July) for placebo-controlled and comparative trials assessing cognition, function, behavior, global change, and safety was made. Thirty-three articles on 26 studies were included in the review of meta-analyses of placebo-controlled data, supporting drugs with modest overall benefits for stabilizing or slowing decline in cognition, function, behavior and clinical global change. Three open-label trials and one double-blind randomized trial directly compared donepezil with galantamine and rivastigmine. The results are conflicting: two studies suggest no differences in efficacy between the compared drugs, while one study found donepezil to be more efficacious than galantamine and the other study found rivastigmine to be more efficacious than donepezil. Adjusted indirect comparison of placebo-controlled data did not find statistically significant differences among drugs with regard to cognition, but found the relative risk of the global response to be better with donepezil and rivastigmine compared with

galantamine. Indirect comparisons also favored donepezil over galantamine with regard to behavior. Across trials, the incidence of adverse events was generally lower for donepezil and the highest for rivastigmine. These studies are discussed in this presentation [30].

Reelin signaling through apolipoprotein E (ApoE) receptors activates a signaling cascade that protects against amyloid-beta (Aβ) at the level of *N*-methyl-d-aspartate receptor (NMDAR) endocytosis, actin polymerization, and tau phosphorylation. ApoE4 induces neuronal resistance to Reelin by impairing the recycling of vesicles containing ApoE receptors, which results in reduced surface expression of the receptors.ApoE receptors on the presynaptic neuron affect spontaneous vesicle release by increasing the mobilization of vesicle-associated membrane protein 7 (VAMP7)-containing vesicles. Astrocytes express ApoE receptors, which may play a role in gliotransmission and synaptic pruning [31]. Utilizing the structure-based drug discovery approach [48], several potent ApoE4 inhibitors from the plant source were identified. The identified compounds were validated computationally and their activity determined against the protein.

The conventional pharmacotherapy of AD employs the use of compounds that inhibit the enzyme acetylcholinesterase (e.g. donepezil, rivastigmine), thereby elevating the levels of Acetylcholine in the nervous tissue of the brain. Lately, another drug has come into the picture for treatment of AD, i.e., memantine. It is a glutamatergic antagonist that protects the nervous tissue against glutamate-mediated excitotoxicity. However, both these classes of drugs provide only the symptomatic relief. There has been a desperate need arising since the past few decades of evolution for a drug that could treat the underlying causes of AD and thereby halt its development in susceptible individuals. There are several plants and derived products that have been employed for their benefits against the symptoms and complications of AD. Some novel drugs having the potential to moderate AD are under clinical trial. This review presents a comprehensive overview of the existing and the upcoming potential treatments for AD [33].

In industrialized countries, AD represents the most devastating neurodegenerative disorder in elderly people and the search for a disease modifying agent is still justified by this unmet need. Several possible targets have been explored to find an appropriate drug therapy, and in this review, dual inhibitors of beta secretase and glycogen synthase kinase 3, recently reported in the literature, will be appraised. Applying a ligand-based approach, the triazinone core emerged as a suitable scaffold to simultaneously bind the aspartic dyad of BACE-1 and the ATP site of GSK-3β, leading to a series of small molecules endowed with a balanced micromolar affinity and a promising pharmacokinetic profile. Differently, by means of a structure-based approach, a series of well-balanced dual binding molecules were designed, taking advantage of the versatility of the curcumin scaffold. For some of these new compounds a potential neuroprotective effect was also observed, due to their ability to counteract the oxidative stress through the inhibition of NQO1 enzyme. Finally, different virtual screening

analyses were performed, leading to the identification of new potential scaffolds deserving further development [43].

10. Drugs, Pharmacotherapy and Pharmaocgenomics of AD

No disease modifying drugs are available for AD, but some options may reduce the symptoms and help improve the quality of life. Cholinesterase inhibitors that are FDA approved drugs for symptomatic relief help individuals to carry out the activities of daily living by maintaining thinking, memory, or speaking skills. They can also help with some of the behavioral and personality changes associated with AD, however, they will not stop or reverse AD and appear to help individuals for only a few months to a few years. The following drugs are prescribed to treat mild to moderate AD symptoms.

- Donepezil (Aricept)
- Rivastigmine (Exelon)
- Galantamine (Razadyne)

10.1. Cholinesterase Inhibitors

⇧ Preventing the breakdown of acetylcholine (a-SEA-til-KOH-lean), a chemical messenger important for learning and memory. This supports communication among nerve cells by keeping acetylcholine levels high.

⇧ Delay or slow worsening of symptoms. Effectiveness varies from person to person.

⇧ If side effects occur, they commonly include nausea, vomiting, loss of appetite, and increased frequency of bowel movements [7].

A different kind of drug, Memantine (Namenda), an NMDA receptor antagonist may also be used, alone or in combination with a cholinesterase inhibitor which is prescribed to treat moderate to severe AD symptoms [1].

10.2. Treatment Using Memantine

This drug regulates the activity of glutamate, a chemical involved in information processing, storage and retrieval. It improves the mental function and ability to perform daily activities for some people. It can cause side effects, including headache, constipation, confusion, and dizziness. Many people are in the hope that supplements such as Vitamin E, Coenzyme Q10, Coral Calcium, Ginko biloba and Huperzine A might work well as treatments for this disease [8].

10.3. Pharmacogenomic Approaches for AD

Pharmacogenomics will be the future therapeutic tool for most genetic disorder worldwide. Screening the individual difference in the genome and finding the suitable drug treatment could produce better recovery. For a decade, the pharmacogenomic research has proven its efficiency in treating various diseases. AD patients have to be screened worldwide to prepare a frequency data and genotyping may prove helpful for the discovery of individualized medicines for the treatment of AD.

11. Conclusion

AD is a neurodegenerative disorder related to aging, characterized by progressive memory loss, cognitive impairment, and the inability to carry out functional activities of daily living. It is characterized pathologically by the presence of cerebral β-amyloid (Aβ), neurofibrillary tangles composed of hyperphosphorylated tau and neurodegeneration. The presence of the apolipoprotein E (ApoE) ε4 allele is the main genetic risk factor associated with sporadic disease, which is the predominant form of AD. Other factors that have been reported to influence the onset of AD include diet as well as physical and mental activity. Significant research attention has focused on the identification of anti-Alzheimer agents for the prevention of AD and preservation in the elderly. In this study, several targets were discussed to conclude the significant targets available to target AD. Each target was highlighted in several molecular pathogeneses that cause AD. Among them, APP, BACE, GSAP, Tau, APOE, PSEN, LRP, and PKCα are the most studied targets and have been considered to have an important role in the pathogenesis of AD. The review has provided the mechanism, the structural organization of each target with their role in AD, and listed out the available inhibitors. Finally, the study concludes by emphasizing that its findings may be beneficial for the treatment of Alzheimer's disease and for future drug designing.

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