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Review

Amyloid beta: structure, biology and structure-based therapeutic development

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Abstract

Amyloid beta peptide (Aβ) is produced through the proteolytic processing of a transmembrane protein, amyloid precursor protein (APP), by β- and γ-secretases. Aβ accumulation in the brain is proposed to be an early toxic event in the pathogenesis of Alzheimer's disease, which is the most common form of dementia associated with plaques and tangles in the brain. Currently, it is unclear what the physiological and pathological forms of Aβ are and by what mechanism Aβ causes dementia. Moreover, there are no efficient drugs to stop or reverse the progression of Alzheimer's disease. In this paper, we review the structures, biological functions, and neurotoxicity role of Aβ. We also discuss the potential receptors that interact with Aβ and mediate Aβ intake, clearance, and metabolism. Additionally, we summarize the therapeutic developments and recent advances of different strategies for treating Alzheimer's disease. Finally, we will report on the progress in searching for novel, potentially effective agents as well as selected promising strategies for the treatment of Alzheimer's disease. These prospects include agents acting on Aβ, its receptors and tau protein, such as small molecules, vaccines and antibodies against Aβ; inhibitors or modulators of β- and γ-secretase; Aβ-degrading proteases; tau protein inhibitors and vaccines; amyloid dyes and microRNAs.

Keywords: amyloid beta peptide; amyloid precursor protein; Alzheimer's disease; neurodegenerative diseases; drug discovery

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Introduction

Alzheimer's disease is the most common type of dementia. It affects tens of millions of people worldwide, and this number is rising dramatically. The social and economic burden of Alzheimer's disease is high. The amyloid hypothesis[1-3] proposes β-amyloid (Aβ) as the main cause of the disease and suggests that misfolding of the extracellular Aβ protein accumulated in senile plaques[4] and the intracellular deposition of misfolded tau protein in neurofibrillary tangles cause memory loss and confusion and result in personality and cognitive decline over time. Accumulated Aβ peptide is the main component of senile plaques and derives from the proteolytic cleavage of a larger glycoprotein named amyloid precursor protein (APP). APP is a type 1 membrane glycoprotein that plays an important role in a range of biological activities, including neuronal development, signaling, intracellular transport, and other aspects of neuronal homeostasis. Several APP cleavage products may be major contributors to Alzheimer's disease, causing neuronal dysfunction. Deposits of Aβ peptides are mainly observed in the region of the hippocampus and the neocortex as well as in the cerebrovasculature (CAA)[5].

As Aβ peptides are the main components of senile plaques, understanding the structures and biochemical properties of Aβ will advance our understanding of Alzheimer's disease at the molecular level. Aβ monomers aggregate into different forms of oligomers, which can then form regular fibrils. The peptides share a common structural motif and aggregation pathway, providing a powerful conceptual framework for understanding the pathogenic mechanism and disease-specific factors. Here, we review the structure and biology of Aβ, which may constitute a core pathway for the growing number of neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, as well as structure-based drug discovery, which may contribute to the development of novel treatment strategies against different degenerative diseases.

Structure of the amyloid beta peptide

Molecular architecture of APP and its proteolysis in the amyloidogenic pathway

The Aβ peptides are cleaved from the much larger precur-

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sor APP. APP is an integral membrane protein expressed in many tissues, especially in the synapses of neurons, which plays a central role in Alzheimer's disease (AD) pathogenesis. APP consists of a single membrane-spanning domain, a large extracellular glycosylated N-terminus and a shorter cytoplasmic C-terminus. It is one of three members of a larger gene family in humans. The other two family members are the APP-related proteins (APLPs) APLP1 and APLP2[6]. APP has been implicated as a regulator of synaptic formation and repair[7], anterograde neuronal transport[8] and iron export[9]. It is produced as several different isoforms, ranging in size from 695 to 770 amino acids. The most abundant form in the brain (APP695) is produced mainly by neurons and differs from longer forms of APP in that it lacks a Kunitz-type protease inhibitor sequence in its ectodomain[10]. APP isoform 695 is mainly expressed in neurons, whereas APP751 and APP770, which contain the Kunitz-type serine protease inhibitory domain KPI, are mainly expressed on peripheral cells and platelets[11, 12] (Figure 1).

APP is best known as the precursor molecule cut by β-secretases and γ-secretases to produce a 37 to 49 amino acid residue peptide, Aβ[13], that lies at the heart of the amyloid cascade hypothesis and whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients. Human APP can be processed via two alternative pathways: amyloidogenic and nonamyloidogenic. APP is first cleaved by α-secretase (nonamyloidogenic pathway) or β-secretase (amyloidogenic pathway), generating membrane-tethered α- or β-C terminal fragments (CTFs). The cleavage of APP by α-secretase releases sAPPα from the cell surface and leaves an 83-amino-acid C-terminal APP fragment (C83). The production of sAPPα increases in response to electrical activity and the activation of muscarinic acetylcholine receptors, suggesting that neuronal activity

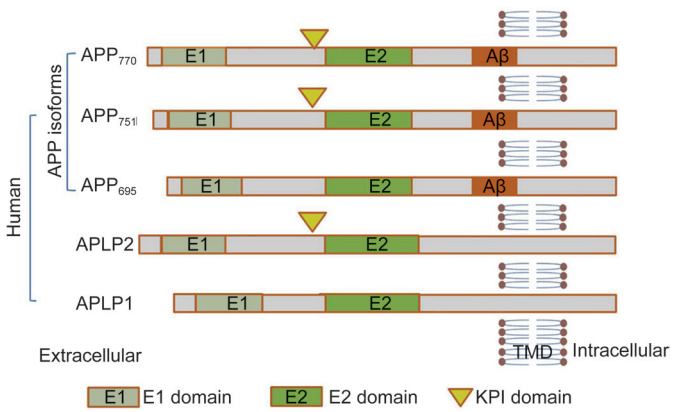


Figure 1. Molecular architecture of APP. Schematic representation of human APP isoforms and the APP-like proteins (APLP), APLP1 and APLP2. APP isoforms range in size from 695 to 770 amino acids. The most abundant form in brain is APP695, which lacks a Kunitz type protease inhibitor sequence in its ectodomain. APP751 and APP770 contain the Kunitz type serine protease inhibitory domain (KPI) are mainly expressed on the surface of peripheral cells and platelets.

increases the α-secretase cleavage of APP [14]. Further processing involves the intramembrane cleavage of α- and β-CTFs by γ-secretase, which liberates the P3 (3 kDa) and Aβ (4 kDa) peptides, respectively[15, 16]. The amyloidogenic processing of APP thus involves sequential cleavages by β- and γ-secretase at the N and C termini of Aβ, respectively (Figure 2)[17]. The 99-amino-acid C-terminal fragment of APP (C99) generated by β-secretase cleavage can be internalized and further processed by γ-secretase at multiple sites to produce cleavage fragments of 43, 45, 46, 48, 49 and 51 amino acids that are further cleaved to the main final Aβ forms, the 40-amino-acid Aβ40 and the 42-amino-acid Aβ42, in endocytic compartments[18, 19]. The cleavage of C99 by γ-secretase liberates an APP intracellular domain (AICD) that can translocate to the nucleus, where it may regulate gene expression, including the induction of apoptotic genes. The cleavage of APP/C99 by caspases produces a neurotoxic peptide (C31)[20]. The β-site APP cleaving enzyme is abundant in neurons, which may accelerate the amyloidogenic processing pathway in the brain and impair neuronal survival. The three-dimensional structure of human γ-secretase was determined by single-particle cryo-electron microscopy in 2014[21]. The γ-secretase complex comprises a horseshoe-shaped transmembrane domain, which contains 19 transmembrane segments (TMs), and a large extracellular domain (ECD) from the nicastrin subunit, which localizes immediately above the hollow space formed by the TM horseshoe. This structure serves as an important basis for understanding the mechanisms of γ-secretase function. The γ-secretase complex consists of four different proteins, presenilin, nicastrin, presenilin enhancer 2 and anterior pharynx-defective 1. Presenilin is activated by auto-processing to generate N- and C-terminal cleavage products that both contain aspartyl protease sites that together are required for the activity of the mature γ-secretase. Nicastrin, presenilin enhancer 2 and anterior pharynx-defective 1 are critical components of γ-secretase and may modulate enzyme activity in response to physiological stimuli[22-24]. This unique cleavage process of APP provides essential targets for AD therapeutics[25].

Aβ monomer

Aβ monomers aggregate into various types of assemblies, including oligomers, protofibrils and amyloid fibrils. Amyloid fibrils are larger and insoluble, and they can further assemble into amyloid plaques, while amyloid oligomers are soluble and may spread throughout the brain. The primary amino acid sequence of Aβ was first discovered from extracellular deposits and amyloid plaques in 1984[2]. The primary amino acid sequence of the 42-amino-acid Aβ isoform $Aβ_{42}$ is shown here (Figure 3A). Aβ encompasses a group of peptides ranging in size from 37 to 49 residues. Amyloid plaques with Aβ as the main component are most commonly found in the neocortex in the brain of Alzheimer's disease patients[26].

Aβ is commonly thought to be intrinsically unstructured and hence cannot be crystallized by common methods. Many studies therefore focus on optimizing conditions that can stabilize Aβ peptides. The three-dimensional solution structure

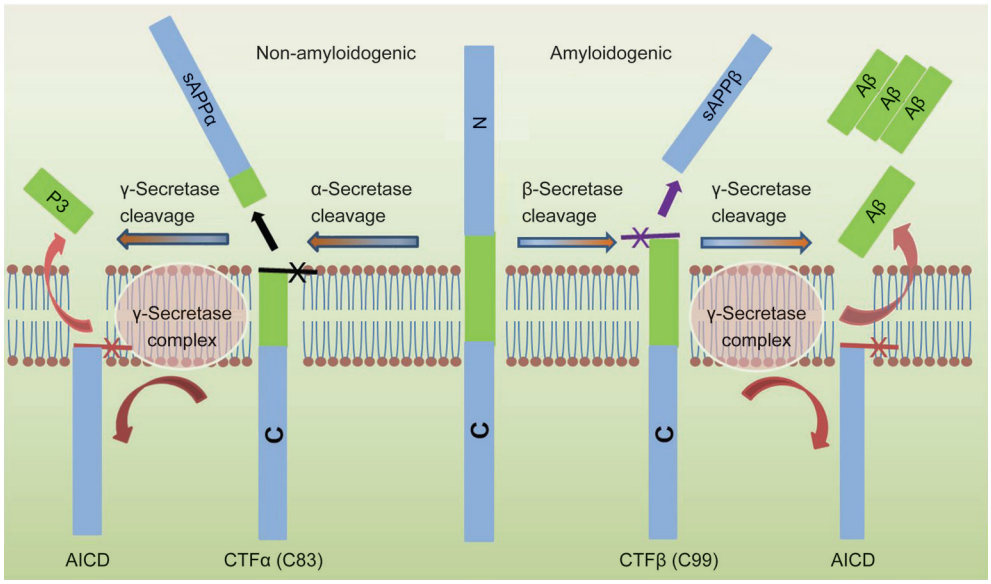


Figure 2. Human APP proteolytic pathways. Human APP proteolysis in the non-amyloidogenic pathway and amyloidogenic pathway. Non-amyloidogenic processing of APP refers to the sequential processing of APP by membrane bound α-secretases, which cleave within the Aβ domain to generate the membrane-tethered α-C terminal fragment CTFα (C83) and the N-terminal fragment sAPPα. CTFα is then cleaved by γ-secretases to generate extracellular P3 and the APP intracellular domain (AICD). Amyloidogenic processing of APP is carried out by the sequential action of membrane bound β- and γ-secretases. β-Secretase cleaves APP into the membrane-tethered C-terminal fragments β (CTFβ or C99) and N-terminal sAPPβ. CTFβ is subsequently cleaved by γ-secretases into the extracellular Aβ and APP intracellular domain (AICD).

of different fragments of the Aβ peptide was determined using nuclear magnetic resonance (NMR) spectroscopy, molecular dynamic (MD) techniques and X-ray crystallography. Most structural knowledge about Aβ comes from NMR and molecular dynamics.

Early NMR-derived models of the solution structure of Aβ peptide (1-28) indicated that it folds into a predominately α-helical structure with β-sheet conversion in membrane-like media that may also occur during the early stages of amyloid formation in Alzheimer's disease[27] (Figure 3B). It is the major proteinaceous component of amyloid deposits in Alzheimer's disease, where the side chains of histidine-13 and lysine-16 residing on the same face of the helix are in close proximity. The solution structure of Aβ peptide (1-40) suggests that the C-terminus of the peptide has an α-helix conformation between residues 15 and 36 with a kink or hinge at 25-27 in aqueous sodium dodecyl sulfate (SDS) micelles, while the peptide is unstructured between residues 1 and 14, which are mainly polar and likely solvated by water. The deprotonation of two acidic amino acids in the helix promotes a helix-to-coil conformational transition that precedes the aggregation of Aβ1-40[28] (Figure 3C). Solid-state NMR spectroscopy-derived models of the solution structure of Aβ peptide (10-35) show that in water[29] (Figure 3D), the peptide collapses into a compact series of loops, strands, and turns without alpha-helical or beta-sheet structure. The van der Waals and electrostatic forces maintain its conformational stabilization. Approximately 25% of the surface is uninterruptedly hydrophobic, and the compact coil structure is meta-stable, which may lead to a global conformational rearrangement and the formation of an intermolecular beta-sheet secondary structure caused by fibrillization. The 3D NMR structures of Aβ peptide (8-25) and Aβ peptide (28-38) show two helical regions connected by a regular type I β-turn. Aβ peptide (25-35) is a highly toxic synthetic derivative of Aβ peptides. Researchers have used NMR and CD investigation of Aβ peptide (25-35) and fluoro-alcohols to scan its conformational properties. The peptide behaves as a typical transmembrane helix in a lipidic environment, forming fibrillar aggregates, which suggests a direct mechanism of neurotoxicity[30, 31].

NMR-guided simulations of Aβ peptides 1-40 (Aβ40) and 1-42 (Aβ42) also suggested very different conformational states[32], with the C-terminus of Aβ42 being more structured and residues 31-34 and 38-41 forming a β-hairpin that reduces the C-terminal flexibility, which may be responsible for the greater propensity of Aβ42 than Aβ40 to form amyloids. Replica exchange molecular dynamics studies suggested that Aβ40 and Aβ42 can indeed populate multiple discrete conformations, comprising α-helix or β-sheet conformers, and the structural states transition rapidly[33]. More recent studies identified a multiplicity of discrete conformational clusters by statistical analysis[34]. However, the most recent NMR structure of Aβ40 shows significant secondary and tertiary structure[35]. The hydrophobic C-terminal of the Aβ is critical in triggering the transformation from α-helical to β-sheet structure and plays a key role in determining the state of protein aggregation in Alzheimer's disease[36].

Aggregation of Aβ into fibrils

Early proposals supported the so-called "amyloid cascade

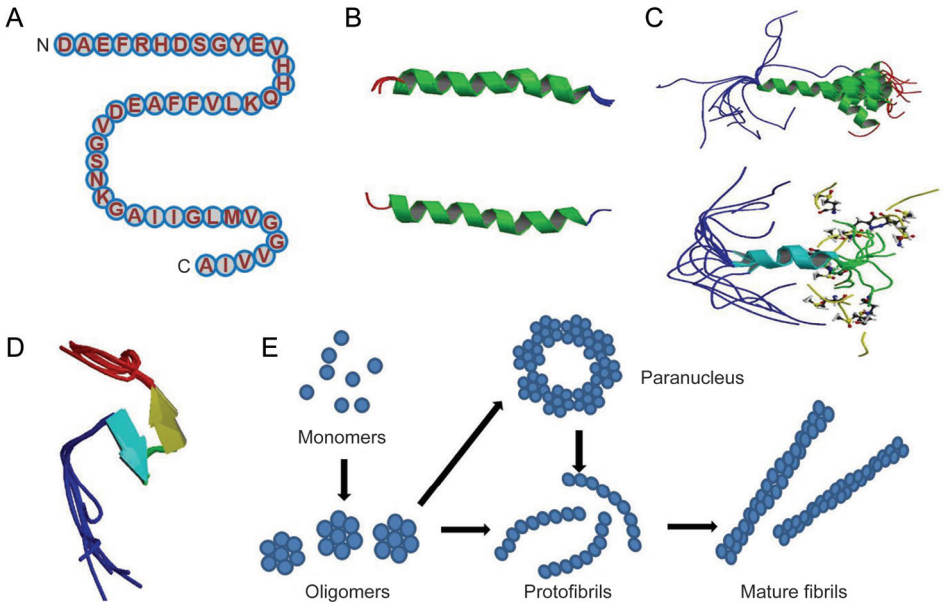


Figure 3. Structures of Aβ monomer, fibril and oligomers. (A) The primary amino acid sequence of the 42 amino acid Aβ isoform Aβ42. Aβ encompasses a group of peptides ranging in size from 37–49 residues. (B) The structure of amyloid beta peptide (1–28), which forms a predominately alpha-helical structure that can be converted to a beta-sheet structure in membrane-like media (PDB code: 1AMC, 1AMB), it's the major proteinaceous component of amyloid deposits in Alzheimer's disease. The side chains of histidine-13 and lysine-16 residing on the same face of the helix are close. (C) Solution structure of amyloid beta peptide (1–40), in which the C-terminal two-thirds of the peptide form an alpha-helix conformation between residues 15 and 36 with a kink or hinge at 25–27 in aqueous sodium dodecyl sulfate (SDS) micelles with a bend centered at residue 12, while the peptide is unstructured between residues 1 and 14 which are mainly polar and likely solvated by water (PDB code: 1BA4, 1BA6). It collapsed into a compact series of loops, strands, and turns with no alpha-helical or beta-sheet structure. The van der Waals and electrostatic forces maintain its conformational stabilization. Approximately 25% of the surface is uninterrupted hydrophobic, and the compact coil structure is meta-stabled, which may lead to a global conformational rearrangement and formation of intermolecular beta-sheet secondary structure caused by fibrillization. (D) Amyloid beta peptide (10–35) forms a collapsed coil structure (PDB code: 1HZ3). It collapsed into a compact series of loops, strands, and turns with no alpha-helical or beta-sheet structure. The van der Waals and electrostatic forces maintain its conformational stabilization. Approximately 25% of the surface is uninterrupted hydrophobic, and the compact coil structure is meta-stabled, which may lead to a global conformational rearrangement and formation of intermolecular beta-sheet secondary structure caused by fibrillization. (E) Proposed pathway for the conversion of amyloid beta monomers to higher order oligomers, protofibrils and fibrils. Aβ monomers can form higher order assemblies ranging from low molecular weight oligomers, including dimers, trimers, tetramers, and pentamers, to mid-range molecular weight oligomers including hexamers, nonamers and dodecamers to protofibrils and fibrils.

hypothesis," which proposes that Aβ aggregation into plaques leads to neurotoxicity and dementia[37] through common cytopathic effects that contribute to the pathogenesis of Alzheimer disease and other amyloidosis. While the Aβ peptide can rapidly aggregate to form fibrils that deposit into the amyloid plaques, which are found to be linked with Alzheimer's disease, later studies demonstrated that there is no direct correlation between amyloid plaques[38] and the loss of synapses and neurons in brains with Alzheimer's disease[39, 40]. Many pathways may lead to the peptide aggregation. Early studies indicated that the amyloid polypeptide is organized in a characteristic "cross β" pattern in a regular manner, in which adjacent chain segments are folded in an anti-parallel manner within the fiber lattice[41]. Later research revealed that the peptide chains of β-strand segments run perpendicular to the long fibril, and the intermolecular hydrogen bonds of β-strands run parallel to the axis in a "cross β" structural pattern[42].

Solid-state NMR measurements have shown that amyloid fibril "cross β" structures exist in two patterns: parallel and antiparallel. The crosslinking of Aβ peptides by tissue transglutaminase (tTg) indicated that the Aβ fibril is a hydrogen-bonded, parallel β-sheet that defines the long axis of the Aβ fibril propagation[43]. Specific amino acid contacts have implications for the overall fibril formation of the extended Aβ (10-35) and its stability, morphology and parallel organization[44]. Multiple quantum (MQ) ^{13}C NMR data indicate an in-register, parallel organization[45]. These measurements, known as experimental EM, STEM, and solid-state NMR, suggest that the supramolecular structures of Aβ peptide (1-40) fibrils, Aβ peptide (10-35), and Aβ peptide (1-42) fibrils are organized as β-sheets[46, 47]. As Aβ peptide aggregation pathways are determined by the primary amino acid sequence and the intermolecular interactions, later studies in the structural organization of disease-related amyloid fibrils have led to the identification of the exact register motif. In addition to the parallel pattern, several short peptide segments of Aβ can adopt an antiparallel pattern[48]. Solid-state NMR spectroscopy indicates amyloid fibrils with a simple and intriguing structural motif[49]. Site-directed spin labeling and electron paramagnetic resonance (SDSLEPR) spectroscopy for amyloid fibrils confirmed that this

parallel, exact register structural motif is highly conserved[50-52]. Progress has been made by disulfide cross-linking within preformed fibrils, with results indicating that they are located proximally inside the hairpin turn. Residues 17 and 34 could be efficiently cross-linked by a disulfide bond, while residues 17/35 and 17/36 were not efficiently cross-linked in fibrils. Purified double mutant proteins consisted of disulfide-bonded monomers that were able to assemble into amyloid fibrils[53]. The 17/35 residues on the C-terminal strand would need to flip 180 degrees to provide the structural flexibility allowing Aβ to assemble into at least two slightly different forms[54-56]. These results are inconsistent with the hairpin model based on electrostatic interactions, with the exception of the side chains of Glu22 and Lys28[57]. It is unclear whether small differences in the fibril structure are pathologically significant; however, the slight two-residue difference in Aβ40 and Aβ42 leads to great differences in their biophysical, biological, and clinical behaviors. The 3D structure of residues 15-42 of Aβ42 adopts a double-horseshoe-like cross-β-sheet entity with maximally buried hydrophobic side chains, in which residues 1-14 are partially ordered and in a β-strand conformation, which is the more neurotoxic species, aggregates much faster, and dominates in senile plaque in Alzheimer's disease patients[58]. Further studies reported that cognitive deficits appeared before plaque deposition or the detection of insoluble amyloid fibrils[59, 60]. In contrast, the amount of oligomeric Aβ[61, 62] is increased in Alzheimer's disease brain extracts[63], which is the basis for the Aβ oligomer hypothesis[64-66], which posits that soluble Aβ oligomers rather than insoluble fibrils or plaques trigger synapse failure and memory impairment[67], resulting in impaired brain function in the final stages of the disease.

Aβ oligomers

While amyloid fibrils are larger, insoluble, and assemble into amyloid plaques forming histological lesions that are characteristic of Alzheimer's disease, Aβ oligomers are soluble and may spread throughout the brain. The size distribution of Aβ oligomers is heterogeneous. There is a broad consensus for the preferential accumulation of a soluble high-molecular-weight species of approximately 100–200 kDa under relatively physiological conditions *in vitro*[68-72]. Aβ monomers can form higher-order assemblies ranging from low-molecular-weight oligomers, including dimers, trimers, tetramers, and pentamers, to midrange molecular weight oligomers, including hexamers, nonamers and dodecamers, to protofibrils and fibrils (Figure 3E). In contrast to the fibril structure, relatively little is known about the structure of amyloid oligomers. Soluble oligomers prepared in the presence of detergents seem to feature substantial beta sheet content with mixed parallel and antiparallel character[73]. The structural characterization of oligomers is complicated because their oligomeric states are more transient than fibrils, and preparing homogeneous populations of oligomers is difficult[74]. They can be stabilized by detergents, which may help to alleviate this problem[75]. There was little structural information on the oligomeric state of amyloid beta until 2010, when low temperature and low salt conditions made it possible to isolate pentameric disc-shaped oligomers devoid of beta structure[76]. Circular dichroism and infrared spectroscopy indicate that Aβ oligomers are extended coil or beta sheet structures[63]. Hydrogen deuterium exchange analysis also indicates that they have a stable core, which is consistent with substantial beta sheet character, as 40% of the total backbone hydrogen bonds are resistant to exchange in the oligomeric conformation with a stable beta sheet secondary structure[77]. In contrast, fifty percent of the backbone hydrogen bonds are resistant to exchange in the mature amyloid fibril, indicating that a small increase in main chain hydrogen bonding accompanies the transition to the fibrillar conformation[78]. Computational studies suggest that Aβ oligomers form an antiparallel beta-turn-beta motif[79]. The solution conformation of Aβ is of significant importance during self-assembly in water environments. The soluble peptide has no alpha-helical or beta-sheet character but adopts a collapsed coil structure[80]. A particular conformation that forms ring-shaped pentamers and hexamers is stable by microsecond all-atom MD simulations[81].

The relationship between oligomers and fibrils remains to be established. There seem to be some similar structural elements, as they both appear to be extended or beta sheet structures and both display similar amounts of main chain hydrogen bonding that is resistant to exchange. On the other hand, amyloid oligomers and fibrils appear to contain mutually exclusive and non-overlapping conformations recognized as generic antibody epitopes that are common to amyloids of different sequences[74, 82]. Oligomers are a kinetic intermediate waxing at early times during the development of fibrils[83]. Different types of soluble amyloid oligomers have a common structure and share a common mechanism of toxicity[63]. It is also unknown whether the oligomer structures represent basic units of amyloid protein that then assemble into fibrils or are just in equilibrium with monomers, which directly form fibrils without intermediate oligomeric structure. Oligomers appear as spherical aggregates at early times and then elongate by the coalescence of spherical subunits with a “bead” appearance, forming the precursor of protofibrils on the pathway to mature fibers. The parallelism between Aβ monomers represents a key organizing principle for amyloid oligomers and may also serve as a common structural motif for amyloid fibrils[71, 84]. Other studies suggest that the spherical oligomers simply dilute the Aβ monomer concentration and may be off-pathway intermediates[85] or that both on-pathway and off-pathway concurrence is possible under special conditions[69].

The structure of Aβ aggregate forms and the aggregation pathways remain challenging research issues, though considerable progress has been made recently. The interactions of Aβ with transition metals have revealed potential pathogenic interactions and structural consequences. Oligomers that may normally be embedded in the membrane bind to transition metals such as Cu, Zn and Fe[86, 87]. Constitutively metal-bound senile plaques play a role in accelerating the aggregation of amyloid beta peptide[88], and the expression of Aβ oligomers may, in turn, regulate metal transition homeostasis[89-91].

NMR data have provided information on the structure of the Aβ-(1-16)-Zn^{2+} complex in aqueous solution. The residues His(6), His (13), and His (14) and the Glu (11) carboxylate were identified as ligands that tetrahedrally coordinate the Zn(II) cation[92].

All these different structures have been generated in different environments and determined by different techniques. The special form of Aβ structures may be not stable or may be stabilized only in a unique solution; they may be similar but not the same as each other; one structure may depict one representative form of Aβ, and all the forms of Aβ may coexist *in vivo*. Aβ forms a myriad of structures in the monomeric and oligomeric states, all of which result in similar fibril structures. Amyloid fibrils of Aβ form a parallel, in-register cross β-sheet structure. The accumulation of Aβ into long, unbranched fibrils is a hallmark of the disease, as is the loss of neurons due to cell death in parallel with the Aβ aggregation process. These new insights into the structures and aggregation pathways may help to uncover the mechanisms of amyloid pathogenesis in degenerative diseases, ultimately leading to new therapeutic strategies to prevent the formation of toxic aggregates (Table 1).

Biological function of amyloid beta

Aβ production

Alzheimer's disease is characterized by abnormal accumulation of the Aβ protein, which is important for memory and cognition, in the brain regions. Aβ is a normal product of the cellular metabolism derived from the amyloid precursor protein (APP). APP is synthesized in the endoplasmic reticulum (ER) and then transported to the Golgi complex, where it completes maturation and is finally transported to the plasma membrane. Mature APP at the plasma membrane is cleaved by the successive action of the β-secretase and γ-secretase to generate Aβ (Figure 2)[93]. The newly generated Aβ either is released to the extracellular space or remains associated with the plasma membrane and lipid raft structures. The binding of Aβ to ganglioside GM1 in the lipid rafts strongly favors Aβ aggregation[94]. The binding of ApoE to Aβ taken up by the cells through receptor-mediated endocytosis mediated by LRP (LDL receptor-related protein), and LDLR regulates aggregation but also the cellular uptake of Aβ[95]. Endocytosed Aβ also has access to other subcellular compartments through the vesicular transport system. Earlier studies pointed to Aβ fibrils as the neurotoxic agent leading to cellular death, memory loss, and other AD characteristics. Over the last two decades, further investigation has suggested that oligomeric or prefibrillar species of the Aβ peptide are the most damaging to neuronal cells. Soluble Aβ can bind to numerous molecules in the extracellular space, including cell surface receptors, metals and cellular membranes.

Aβ binding receptors

The extracellular accumulation of Aβ in neuritic plaques and the binding of Aβ to a variety of receptors appear to be the characteristic hallmarks of Alzheimer's disease. The binding of Aβ to a variety of receptors has been proposed as a cause for the neuronal toxicity: Aβ oligomers were proposed to induce mitochondrial dysfunction and oxidative stress in AD neurons, resulting in a massive calcium influx and toxicity in neurons[96]. Furthermore, soluble oligomeric Aβ was proposed to be toxic through binding to a variety of receptors, including lipids, proteoglycans, and proteins, such as the Aβ-binding p75 neurotrophin receptor (P75NRT), the low-density lipoprotein receptor-related protein (LRP), cellular prion protein (PrP^c), metabotropic glutamate receptors (mGluR5), α subunit containing nicotinic acetylcholine receptor (α7nAChR), N-methyl-D-aspartic acid receptor (NMDAR), β-adrenergic receptor (β-AR), erythropoietin-producing hepatoma cell line receptor (EphR), and paired immunoglobulin-like receptor B (PirB)[97]. The Aβ/Aβ receptor interactions are proposed to generate and transduce neurotoxic signals into neurons, causing cellular defects such as mitochondrial dysfunction and the ER stress response. In addition, some Aβ receptors are most likely to internalize Aβ into neurons to display distinct cellular defects (Figure 4).

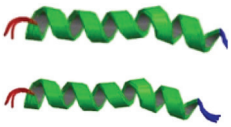
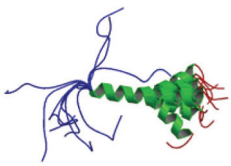
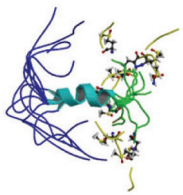
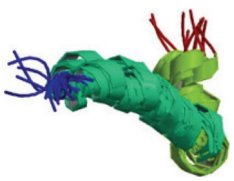
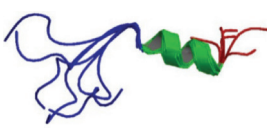
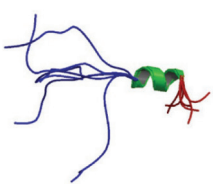
NMDAR and α7nAChR

NMDAR and α7nAChR are both ion channel receptors. Several reports suggest that Aβ interacts with NMDARs at postsynaptic terminals, and antibodies raised against the GluN1 or GluN2B subunit of NMDARs markedly block the binding of the Aβ oligomer to neurons[98, 99], indicating that Aβ oligomers partially co-localize with the GluN2B subunits of NMDARs at the cell surface[100]. Indeed, Aβ directly or indirectly binds to NMDAR subunits to activate NMDAR, and thus Aβ oligomers induce calcium dysregulation, neuronal death [101], and synaptic dysfunction[102, 103]. Moreover, Aβ oligomers promote the endocytosis of NMDARs, which requires the activation of α7nAChR signaling[104]. The receptor α7nAChR is another candidate Aβ-binding receptor and binds to soluble Aβ with high affinity[105]. The α7nAChR-expressing cells are susceptible to Aβ-induced toxicity *in vitro*[106], and it mediates Aβ-induced tau phosphorylation via the ERK and JNK pathways[107]. In a mouse model of AD, α7nAChR may exacerbate AD pathology in a mouse model, while its deficiency may improve cognitive deficits and synaptic pathology[108].

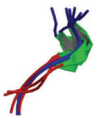
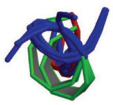
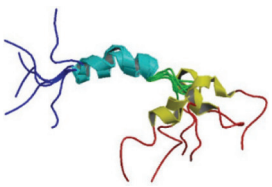
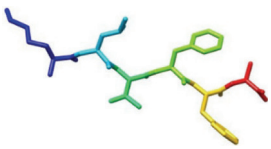
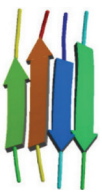
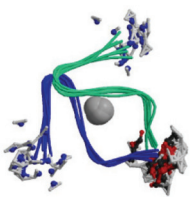
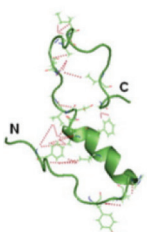
Aβ binding p75 neurotrophin receptor (p75NTR)

The receptor p75NTR is a TNF family low affinity receptor for neurotrophins. Aβ binds to both p75NTR monomers and trimers (Figure 4), which activates their intracellular signaling to induce apoptosis in human neuroblastoma cells. Early studies compared neuroblastoma cell clones that either did not express any of the neurotrophin receptors or had been engineered to express full-length or various truncated forms of the p75NTR[109]. These studies showed that p75NTR binds to Aβ via its extracellular domain, which directly signals cell death via its death domain. In fact, this signaling leads to the activation of caspase 8 and caspase 3 and to the production of reactive oxygen species (ROS) and cellular oxidative stress[110]. In addition, Aβ can interact synergistically with cytokines TNFα

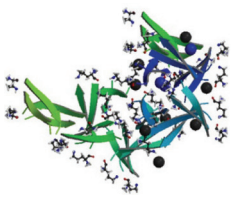
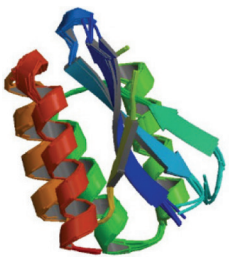
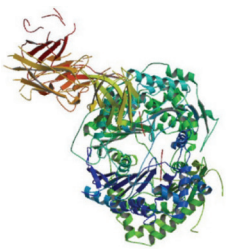
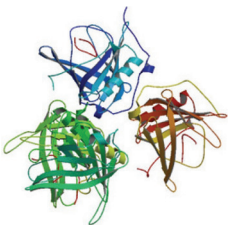
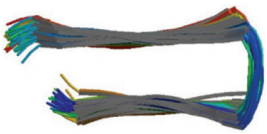
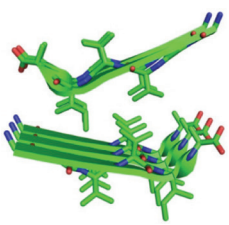
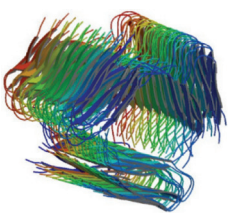
Table 1. Summary of Aβ structural studies.

Proteins and peptides	Structure	Characteristic	PDB code
Aβ1-28		Monomer; NMR; In membrane-like media the peptide folds to form a predominately alpha-helical structure with a bend centered at residue 12.	1AMC 1AMB
Aβ1-40 with Met(O)		Monomer; NMR; forms C-terminal alpha-helix; two acidic amino acids promote a helix-coil conformational transition.	1BA4
			1BA6
Aβ10-35		Monomer; NMR spectroscopy; Aβ collapsed into a compact series of loops, strands, and turns and the absence of alpha-helical or beta-sheet structure in water.	1HZ3
Aβ1-42		Monomer; NMR; two helical regions encompassing residues 8-25 and 28-38, connected by a regular type I beta-turn.	1IYT
Two mutants (K16E, K16F) of Aβ1-28		Monomer; NMR spectroscopy; the two mutations may stabilize the helix and also influence aggregation and fibril formation.	1BJB
			1BJC
Rat Aβ1-28 and its interaction with zinc		Monomer; NMR spectroscopy; A helical region from Glu16 to Val24 exists; Arg13, His6, His14 residues provide Zn^{2+}-binding sites; Zn^{2+}-binding is more stable.	1NMJ

(To be continued)

Proteins and peptides	Structure	Characteristic	PDB code
Aβ25-35		Monomer; CD and NMR; Aβ (25-35) is highly toxic and forms fibrillar aggregates.	1QWP
			1QXC
			1QYT
Aβ1-42		Monomer; CD and Solution NMR; Alpha helix embedded in membrane, beta-sheet structures of amyloid fibrils	1Z0Q
Aβ16-21		X-Ray; fiber-forming segments of Aβ. Self-complementing pairs of β-sheets termed steric zippers.	2Y29
Aβ35-42		Polymorphic oligomers, protofibers, and fibers; Homo tetramer-A4; β-sheets termed steric zippers.	2Y3L
Aβ1-40		Rat homo dimer-A2; Solution NMR; zinc-binding domain formed by residues 1-16 of Aβ.	2LI9
Aβ1-40	 	Monomer; Solution NMR; 3_{10}-helix from H13 to D23 and the N- and C-termini collapse against the helix.	2LFM

(To be continued)

Proteins and peptides	Structure	Characteristic	PDB code
Aβ17-36		Homo 16-mer-A16; X-Ray; Crystallizes to form trimers that further assemble into oligomers; Trimers consist of three β-hairpins; Two trimers form hexamer; four trimers form dodecamer, and 5 dodecamers form an annular pore.	5HOW
Aβ1-40 in complex with affibody protein Z (Aβ3)		Homo trimer-A3; Solution NMR; Z(Aβ3), nanomolar affinity, Bound Aβ(1-40) features beta-hairpin comprising residues 17-36	2OTK
Aβ1-40 in complex with Fab-bound human Insulin Degrading Enzyme (IDE)		Hetero trimer-ABC; X-Ray	4M1C
Aβ1-40 complex with an engineered lipocalin (Anticalin H1GA)		X-Ray	4MVI 4MVK 4MVL
Aβ1-42 fibrils		Homo pentamer-A5; Solution NMR; residues 18-42 form intermolecular parallel beta-strand-turn-beta-strand motif	2BEG
Aβ37-42 fibrils		Homo tetramer-A4; X-Ray; a pair of beta-sheets, with the facing side chains of the two sheets interdigitated in a dry 'steric zipper'	2ONV
Aβ fibrils		Fibrils; solid-state NMR; the fibril backbone arrangement, stacking registry, and "steric zipper" core interactions	2MPZ

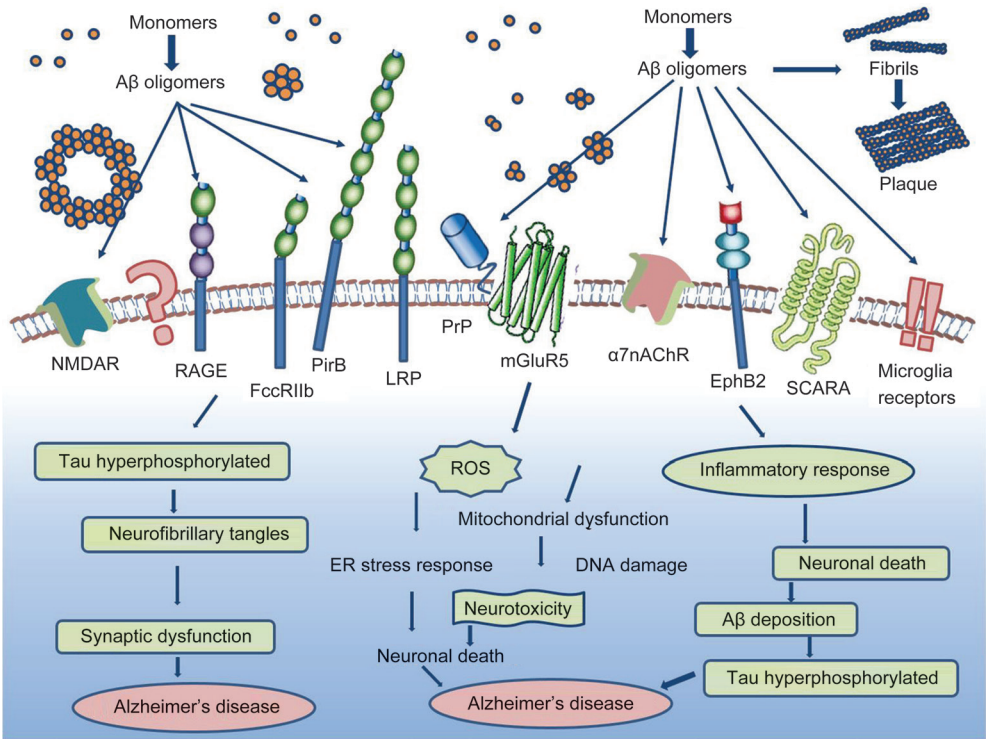


Figure 4. Biological functions of Aβ. Aβ monomers can form higher order assemblies ranging from low molecular weight oligomers (including dimers, trimers, tetramers, and pentamers) to midrange-molecular weight oligomers, high molecular weight oligomers, protofibrils fibrils and senile plaques. Soluble Aβ can interact with potential receptors and activate downstream pathways to generate reactive oxygen species, hyperphosphorylate Tau protein, and cause inflammatory responses, which may result in neuronal death and lead to Alzheimer's disease.

and IL1β, which markedly strengthens the neurotoxic actions of Aβ/p75NTR signaling and potentiates neuronal damage. Aβ-bound p75NTR triggers cell death in the hippocampus of human Alzheimer's disease brains. Taken together, these findings indicated that p75NTR-expressing neurons endowed with receptors for proinflammatory cytokines might be the reason for the target selectivity of Aβ cytotoxic actions in Alzheimer's disease[111, 112].

Low-density lipoprotein receptor-related protein (LRP)

The low-density lipoprotein receptor-related protein (LRP), also known as alpha-2-macroglobulin receptor (A2MR), apolipoprotein E receptor (APOER) or cluster of differentiation 91 (CD91), is a protein receptor found in the plasma membrane of cells involved in receptor-mediated endocytosis. LRP1 is involved in various biological processes such as lipoprotein metabolism and cell motility, and pathologically in neurodegenerative diseases, atherosclerosis and cancer[113].

LRP is a multifunctional cell surface receptor of more than 600 kDa in size with a single transmembrane-spanning domain. LRP has more than 20 identified ligands, many of which are localized to the central nervous system. The broad categories of these ligands include apolipoprotein E (apoE) and lipid-related ligands as well as protease and protease inhibitor complexes such as APP containing Kunitz proteinase inhibitor, α2M, tissue plasminogen activator and plasminogen activator inhibitor 1 complexes, and others such as lactoferrin. Cholesterol is imported into neurons by apoE via LRP1 receptors. Starving neurons of cholesterol and malfunction of the neuronal cholesterol metabolism is thought to be a causal factor in Alzheimer's disease[114]. In addition, overaccumulation of copper in the brain is associated with reduced LRP1-mediated clearance of Aβ across the blood brain barrier. This defective clearance may contribute to the buildup of neurotoxic Aβ[115]. Together, these studies support a critical role of the multifunctional receptor LRP in Aβ metabolism and Alzheimer's disease.

LRP also interacts with the amyloid precursor protein itself. LRP regulates APP trafficking and processing by different mechanisms. SorLA (also called SORL1, SORLA1, or LR11) is a neuronal apolipoprotein E receptor that can regulate the intracellular transport and processing of the APP in neurons. It alters the localization of APP to discrete intracellular compartments, resulting in a decrease of extracellular Aβ levels[116]. LRP and LRP1B expression and endocytosis are thought to play opposing roles in APP endocytosis, resulting in increased APP processing to Aβ levels in the presence of LRP for a rapid fast endocytosis rate and decreased Aβ production in the presence of LRP1B for a slower endocytosis rate[117].

PrP^c and mGlu5 receptors in astrocyte upregulation by Aβ

PrP^c is a glycosylphosphatidylinositol (GPI)-anchored membrane protein that can undergo a conformational change to an infectious, pathological state called scrapie prion protein

(PrP^{Sc}), which is linked to transmittable spongiform encephalopathies and causes terminal neurodegenerative disorders [118]. PrP^{c}-binding ligands include the laminin γ1-chain, Cu^{2+} ions and Aβ42 oligomers[119, 120], the latter of which binds PrP^{c} with high affinity[121].

Metabotropic glutamate receptors (mGluR5) are members of the G-protein coupled receptor superfamily. mGluR5 has been specifically implicated in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease[122-124]. The activation of mGluR5 has been shown to decrease the fragile X mental retardation protein (FMRP)-mediated translation repression of APP and to stimulate sAPPα secretion, which induces the β-secretase pathway and Aβ production. FMRP can stimulate neural pruning and synaptic plasticity, which results in neuroprotection under normal physiological conditions[125]. More recently, mGluR5 has been suggested to be the primary co-receptor for both PrP^{c} and Aβ oligomers[126] (Figure 4). The extracellular domain of mGluR5 interacts with both PrP^{c} and Aβ42, which results in the activation of Ca^{2+} release from intracellular stores, thus promoting PKC translocation and ERK1/2 phosphorylation. Aβ42 and PrP^{c} also activate mGluR5 to stimulate Fyn kinase-mediated APP protein translation.

Immune globin receptors FccRIIb and PirB

Two immune globin receptors, FccRIIb and PirB, originally believed to function exclusively in the immune system, were recently shown to play neuropathic roles as Aβ receptors in Alzheimer's disease brains[97, 127, 128]. These two proteins show similarity in their structures and in the high binding affinity for Aβ oligomers. Both have immunoglobulin (Ig) domains in their extracellular domains and immunoreceptor tyrosine-based inhibitory (ITI) motifs in their intracellular domains. FccRIIb has two Ig domains and one ITI motif, whereas PirB has six Ig domains and four ITI motifs. FccRIIb interacts with low-molecular-weight oligomers via its second Ig domain, and PirB binds to high-molecular weight-oligomers via its first two Ig domains.

Other receptors

Other receptors, such as microglia receptors, are also involved in the amyloid cascade. Microglial membrane receptors bind Aβ and contribute to microglial activation and Aβ phagocytosis and clearance. These receptors can be categorized into several groups. The scavenger receptors (SRs) include scavenger receptor A-1 (SCARA-1), MARCO, scavenger receptor B-1 (SCARB-1), CD36 and the receptor for advanced glycation end product (RAGE)[129]. The G-protein coupled receptor (GPCR) group includes formyl peptide receptor 2 (FPR2) and chemokine-like receptor 1 (CMKLR1)[130], and the toll-like receptor (TLR) group includes TLR2, TLR4, and the co-receptor CD14[131]. Functionally, SCARA-1 and CMKLR1 participate the uptake of Aβ, and RAGE is responsible for the activation of microglia and production of proinflammatory mediators in response to Aβ binding. CD36, CD36/CD47/α6β1-integrin, CD14/TLR2/TLR4, and FPR2 display functions in both Aβ binding and microglia activation. In addition, MARCO and SCARB-1 exhibit the ability to bind Aβ and may be involved in the progression of Alzheimer's disease[132].

A variety of microglia receptors are involved in Aβ clearance and in triggering an inflammatory response. Some receptors, including RAGE and NLRP3, are mainly implicated in the generation of an inflammatory response by triggering a signaling cascade that results in the production of proinflammatory mediators[133]. Other receptors, such as SR-AI and TREM2, participate the clearance of Aβ by inducing internalization of Aβ fibrils. Complement receptors, Fc receptors, FPRL1/FPR2, CD36, and TLRs are involved in both Aβ clearance and the generation of inflammatory responses, while the microglia receptor CD33 seems to accelerate Aβ accumulation.

Aβ degradation

The production of Aβ is normally counterbalanced by several processes, including proteolytic degradation, cell-mediated clearance (which may itself involve proteolytic degradation), active transport out of the brain, and deposition into insoluble aggregates. A growing body of evidence suggests that proteolytic degradation is a particularly important determinant of cerebral Aβ levels and, by extension, of Aβ-associated pathology[134]. The individual Aβ-degrading proteases neprilysin, endothelin-converting enzymes, insulin-degrading enzyme, plasmin and other Aβ-degrading proteases play important roles in Aβ degradation and Alzheimer's disease, although their relative importance remains to be established (Figure 5).

Neprilysin (NEP) is a 93-kDa zinc metallo-endopeptidase implicated in the degradation of a wide array of bioactive peptides[135] and is the most efficient Aβ peptidase. It is a type 2 membrane glycoprotein with its active site located in the intraluminal/extracellular space, into which Aβ peptides are normally secreted[136]. NEP is also localized to the early Golgi and endoplasmic reticulum and other subcellular compartments. Synthetic Aβ was first demonstrated to undergo proteolysis by NEP, and Aβ degradation was most strongly inhibited by a potent and selective inhibitor of NEP, thiorphan[137]. The overexpression of NEP resulted in a lack of amyloid accumulation in APP transgenic mice, while the absence of NEP expression resulted in amyloid aggregation in APP transgenic mice[138, 139]. These experimental and clinical observations, therefore, support the hypothesis that Aβ degradation and the development of idiopathic Alzheimer's disease may be greatly affected by the regulation of an aging-induced reduction of NEP activity.

Endothelin converting enzymes 1 and 2 (ECE1 and ECE2) are membrane-bound zinc metalloproteinases belonging to the same family as NEP (M13 family). While other members of the M13 family are capable of degrading Aβ in cell culture or *in vitro*[140, 141], the addition of phosphoramidon, a known inhibitor of ECEs, can significantly elevate the secretion of Aβ into the medium of cultured cells. The overexpression of ECEs in cultured cells stably expressing APP led to a reduction of more than ninety percent in the level of secreted Aβ, and this effect was reversed by treatment with phosphoramidon. Taken together, the involvement of ECEs may play a causal role in

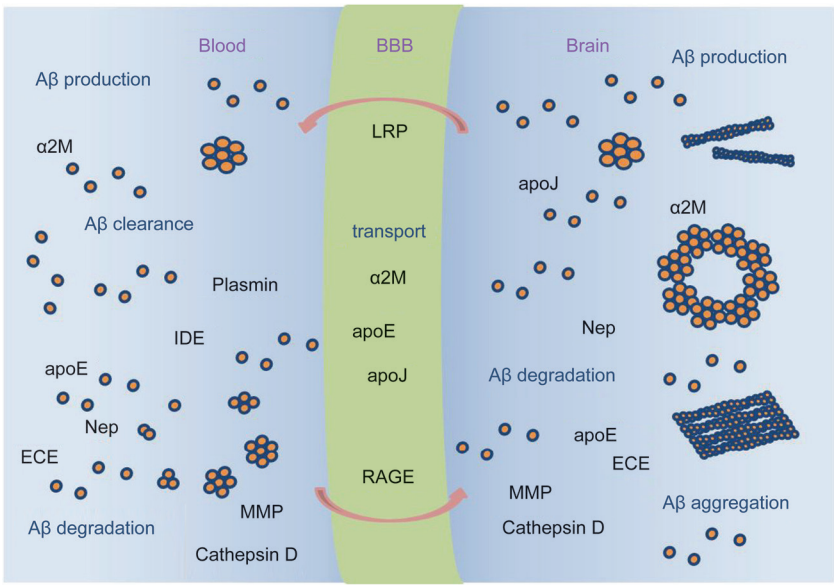


Figure 5. Aβ homeostasis involves production, aggregation, transport, degradation, and clearance. Aβ is produced in peripheral tissues and the CNS, where it can aggregate and form insoluble fibrils. Soluble Aβ can be transported across the BBB from blood to brain via RAGE, and from brain to blood via LRP. Aβ can also bind to transport proteins, *eg*, apoE, apoJ, α2-macroglobulin (α2M), which may influence Aβ sequestration as well as the form of its accumulation in brain. Aβ can be proteolytically degraded by the proteases Neprilysin (Nep), endothelin converting enzymes (ECE), insulin degrading enzyme (IDE), plasmin and other Aβ-degrading proteases (MMP, Cathepsin D), as well as by microglia-mediated degradation.

the degradation of Aβ[142].

Insulin-degrading enzyme (IDE) is a 110-kDa zinc metalloendopeptidase that degrades a broad range of substrates, including insulin, glucagon, and amylin, along with a range of other bioactive peptides[143], as well as the intracellular domain of APP[144]. Early studies identified IDE as the first protease to degrade Aβ *in vitro* within crude brain homogenates[145], and IDE was later identified independently as the major Aβ degrading component secreted into the medium by a range of cultured cells[146]. Moreover, IDE might indirectly affect Aβ levels via its effects on AICD levels, which has recently been implicated in the transcriptional regulation of APP[147] and neprilysin expression[148-149].

Plasmin is a serine protease that is the ultimate effector in the fibrinolytic cascade. Plasmin can degrade and reduce the toxicity of both monomer and fibril Aβ[150-153].

Other candidate Aβ-degrading proteases remain to be identified. The matrix metalloproteases (MMPs) MMP2 and MMP9[154, 155] have been shown to degrade Aβ *in vitro*. Angiotensin-converting enzyme is yet another metalloprotease that may play an important role in the pathogenesis of Alzheimer's disease and that has been shown to degrade Aβ *in vitro*[156]. Cathepsin D, an aspartyl protease localized within lysosomes and endosomes, was identified as a major Aβ-degrading enzyme in brain homogenates[157], and its expression level in the brain is altered in Alzheimer's disease[158].

Aβ transport

In addition to degradation, Aβ released into the extracellular space can be transported between different compartments, such as from the brain to the blood or from the blood to the brain, and can also be cleared by chaperones, such as apoE, which can affect Aβ metabolism after it is released by cells and influence Aβ aggregation, clearance, and transport[159].

The carrier- and receptor-mediated transport of Aβ across the blood brain barrier (BBB) regulates brain Aβ levels[160-165]. The concentration of soluble Aβ in the CNS, which is central to the formation of neurotoxic oligomeric Aβ species[166] and vascular aggregated forms of Aβ, is critically influenced by Aβ transport exchange across the BBB. This transport process has been reported to be regulated by receptors, such as advanced glycation end products (RAGE)[167], or the low-density lipoprotein receptor-related protein 1 (LRP1)[168, 169]. Moreover, other receptors such as glycoprotein 330 (gp330/megalin)[170] and P-glycoprotein[171] may also contribute to the transport of Aβ across the BBB, and the Aβ-binding proteins α2-macroglobulin, apoE and apoJ appear to influence this process[172]. The levels and form of Aβ may be greatly determined by not only the vascular clearance and BBB transport of Aβ, proteolytic degradation[173, 174], oligomerization, and aggregation but also by the production[175, 176] and clearance of different forms of Aβ (fibrillar *vs* soluble) by other cells of the neurovascular unit, such as astrocytes[177-179]. These activities may also play a major role in determining the brain accumulation and associated neuronal and vascular toxicity. Plasma Aβ levels may contribute more to Alzheimer's disease toxicity in cognitively normal elderly individuals[180, 181]. Strategies to clear Aβ from the vascular system can reduce the Aβ levels and amyloid load in the CNS. These strategies include the use of an anti-Aβ antibody[182-184], non-immune approaches with gelsolin, GM1[185], sRAGE[186] or soluble forms of LRP-1, sLRP-1 fragments[187] and insulin-like growth factor I[187]. Many receptors

are involved in inducing Aβ transport and clearance. Among them, RAGE is an influx transport receptor that binds soluble Aβ and mediates pathophysiological cellular responses[188-190]. RAGE also mediates the transport of plasma Aβ across the BBB. LRP-1 functions as a clearance receptor for Aβ at the BBB[191, 192] (Figure 5).

ApoE can regulate Aβ transport, clearance, and aggregation. ApoE is a 299-amino-acid lipid transport protein expressed as three different isoforms: apoE2, apoE3 and apoE4. E3 is the most common isoform, and E4 is responsible for a genetic predisposition to Alzheimer's disease, increasing the risk of Alzheimer's disease by approximately 3-fold more than the E3 allele, whereas E2 decreases AD risk[193]. Glial cells such as astrocytes and microglia secrete apoE into the interstitial fluid (ISF) of the brain. When Aβ is secreted into the brain ISF, mostly by neurons, apoE-containing high-density lipoproteins (HDL) interact with Aβ and influence its clearance into cells via the endocytic LDL receptor family member LDLR. The binding of apoE/Aβ complexes to heparin sulfate proteoglycans (HSPG) can increase the retention of Aβ in the extracellular matrix of the brain and arterioles. This process may play a role in the development of cerebral amyloid angiopathy (CAA)[194]. ApoE and Aβ have been shown to colocalize in detergent-insoluble glycolipid-rich membrane domains (DIGs), which may promote their interaction. In the ISF, apoE/Aβ interactions likely determine whether and when Aβ will aggregate. ApoE may also play a role in Aβ transport out of the brain via ISF/bulk flow, which can modulate both soluble and fibrillary Aβ clearance as well as transport and fibrillogenesis and, in doing so, plays an important role in Alzheimer's disease and CAA pathogenesis[195].

Aβ forms and their toxicity

The different forms of Aβ include soluble Aβ, Aβ oligomer and Aβ present in amyloid plaques. In addition, a dynamic compartmentalization of the different types of Aβ may exist between plaques and soluble Aβ[196], and the different Aβ forms may contribute to neurodegeneration at different stages of the disease[197]. Aβ has also been reported to form aggregates in two fundamental types of reactions: non-metal-dependent association and metal-dependent association. Non-metal Aβ aggregates form soluble oligomers and amyloid fibrils, while metal Aβ aggregates form ionically bridged aggregates, covalently crosslinked oligomers, and seeds for non-metal-dependent Aβ fibrillization[198]. Accumulating Aβ first forms Aβ oligomers and gradually deposits as fibrils and senile plaques. In addition, tau protein becomes hyperphosphorylated in response to kinase/phosphatase activity changes mediated by Aβ aggregation, leading to the formation of neurofibrillary tangles (NFTs), neuronal and eventual synaptic dysfunction, and finally Alzheimer's disease (Figure 4). When the process of self-aggregation occurs on neuron membranes, it generates a toxic aldehyde called 4-hydroxynonenal and leads to lipid peroxidation, which can damage the function of ion-motive ATPases, glucose transporters and glutamate transporters. In turn, Aβ promotes depolarization of the synaptic membrane, excessive calcium influx and mitochondrial damage, which impairs the ability of cells to conduct normal physiological activities[79].

Furthermore, the aggregation of Aβ may also produce free radicals as ROS that react rapidly with proteins or lipids, resulting in the formation of "toxic" oxidized proteins and peroxided lipids. Oxidized proteins are harmful to the membrane integrity and may also alter the sensitivity to oxidative modifications of enzymes such as glutamine synthetase (GS) and creatine kinase (CK), which are critical to neuronal function[199, 200]. Peroxidized lipids can generate toxic products such as 4-hydroxy-2-nonenal (HNE) and 2-propenal (acrolein) that migrate to different parts of neurons and cause multiple harmful alterations to cellular activity. The deleterious functions associated with neuronal death include the inhibition of ion-motive ATPases and glial cell Na^+-dependent glutamate transport, loss of Ca^{2+} homoeostasis, and disruption of signaling pathways[201-203]. In addition to proteins and lipids, oxidative stress induced by Aβ aggregation has also been reported to cause DNA oxidation, leading to DNA damage.

Sustained elevation of Aβ levels and continuous aggregation might also promote a chronic response of the innate immune system by activating microglia, which can lead to neuronal loss through direct phagocytosis. The immunological receptors that are activated by Aβ include toll-like receptor 2 (TLR2), TLR4, TLR6, and their co-receptors CD14, CD36, and CD47[204, 205]. In addition, Aβ aggregation also causes inflammatory responses and the release of inflammation-related mediators, such as eicosanoids, chemokines, proinflammatory cytokines and complement factors, which can increase neuronal death and the loss of neuronal synapses and impair the clearance of Aβ and the neuronal debris mediated by microglia. In addition to the microglia driven neuroinflammatory response[206], this processes is probably also mediated indirectly by regulating kinase/ phosphatase activity.

Moreover, when the Aβ precursor APP accumulates at the mitochondrial membrane, it blocks the translocation of inner mitochondrial metabolites and proteins, leading to disruption of the electron-transport chain and mitochondrial dysfunction, which may in turn increase excessive Aβ generation and result in greater toxicity in a feed-forward loop[207, 208]. Excessive Aβ levels also activate the mitochondrial fission proteins Fis1 and Drp1, thereby inducing mitochondrial fragmentation[209]. Aβ localized in the mitochondria can interact with the proapoptotic factors Aβ-binding alcohol dehydrogenase (ABAD) and cyclophilin D (CypD), resulting in increased neuronal cell death[210]. Therefore, there may be a vicious feedback loop between increased Aβ production and mitochondrial dysfunction.

Extracellular deposits of fibrils or amorphous aggregates of Aβ peptide form plaques and diffuse deposits, while intracellular fibrillar aggregates of hyperphosphorylated and oxidated tau can form neurofibrillar tangles. These plaques and neurofibrillary tangles are deposited mainly in brain regions, such as the hippocampus, amygdala, entorhinal cortex, and basal forebrain, that influence memory and learning and emotional behaviors. Aβ can damage synapses and neurites, and plaque

deposits in brain regions reduce the number of synapses. Aβ specifically damages neurons that produce serotonin and norepinephrine or that employ glutamate or acetylcholine as neurotransmitters. After synthetic Aβ fragments were found to kill cultured neurons[211], the chemical and cell biological bases for the synaptic dysfunction and death of neurons in Alzheimer's disease were reported by a series of studies. Aβ, particularly in its aggregating forms, can impair synaptic ion and glucose transporters, and electrophysiological studies have shown that Aβ impairs synaptic plasticity. Decreasing sAPPα levels can increase the resistance of neurons to oxidative and metabolic insults, which is consistent with sAPPα contributing to the demise of neurons, which is coincident with the increased production of Aβ in Alzheimer's disease[212, 213]. Memory deficits correlate with the formation of Aβ oligomers, which appear relatively early in the process of Aβ deposition in APP mutant mice[214]. Remarkably, the immunization of APP mutant mice with human Aβ42 resulted in the removal of Aβ deposits from the brain and the reversal of cognitive deficits, adding to the evidence that Aβ deposition is a pivotal event in Alzheimer's disease[215].

Aβ and Alzheimer's disease

The cause of most Alzheimer's cases is still unknown. Although it is characterized mostly by the formation of amyloid plaques in the brain, there are several other competing hypotheses regarding the cause of the disease.

The amyloid hypothesis proposed that the fundamental cause of the Alzheimer's disease is the deposits of extracellular Aβ peptides[216]. Mutations in the human *APP* gene cause the development of amyloid plaques and Alzheimer's-like brain pathology, especially in early-onset familial Alzheimer's disease (EOFAD)[217, 218]. Mutations in the human *APP* gene are close to the γ-secretase site and can increase the Aβ42/Aβ40-ratio. It is reported that mutations that alter residues C-terminal to the Aβ42 site reduce cleavage efficiency and increase the Aβ42/Aβ40 ratio[219]. AD-causing mutations also occur in the genes *PSEN1* and *PSEN2*. Mutations in the human *PSEN1* and *PSEN2* genes affect γ-secretase activity and can increase the Aβ42/Aβ40 ratio. Some early-onset families do not show mutations in *APP*, *PSEN1* or *PSEN2*. Several additional key proteins may be involved in γ-and β-secretase cleavage events, as well as in the hyperphosphorylation of tau and the development of neurofibrillary tangles[65, 220]. Late-onset Alzheimer's disease (LOAD) is characterized by a pattern of interwoven genetic and non-genetic factors. These risk-factor genes each affect one or more of the known pathogenic mechanisms: increased Aβ production and aggregation; decreased Aβ clearance and degradation; increased inflammation; and resistance to γ-secretase activity, and thus lead to neurodegeneration in AD[221]. Among these risk genes, for *APOE*, the alleles occurring at the *APOE* loci ε2, ε3 and ε4 were tested and shown to be associated with increased risk of AD[222, 223]. Other researchers have been led to suspect that non-plaque Aβ oligomers are toxic and might be the main cause of neurodegenerative disorders such as Alzheimer's disease[224].

Non-Aβ hypothesis

The cause of most Alzheimer's cases is still unknown. Numerous reports on genetic evidence suggest that Aβ and its aggregation in senile plaques play an important role in the pathogenesis of AD. Aβ cleavage by β-secretase and γ-secretase from APP can result in oligomers that form higher-order fibrils, which then give rise to Aβ plaques. However, genetic causes only explain the small proportion (1% to 5%) of AD cases in which genetic differences have been identified[225]. The dominant mutations in the genes *APP*, *PSEN1* and *PSEN2*[226], which are implicated in AD pathology, are only present in a very small portion of Alzheimer's cases. It has also been reported that the accumulation of the more insoluble Aβ42 over Aβ40 is an important trigger for AD pathogenesis, while APP can alternatively be cleaved by α-secretase to generate non-plaque-forming extracellular peptides in the non-amyloidogenic processing pathway[227]. Our previous studies have tested the effect of all 28 FAD-linked C99 mutations and found that most familiar Alzheimer's disease (FAD)-linked APP mutant proteins cause partial resistance to γ-secretase cleavage[228]. Among them, only mutations that affect residues C-terminal to the Aβ42 cleavage site (Aβ42-53) markedly affect cleavage efficiency and increase the Aβ42 production that leads to AD.

AD pathogenesis includes both Aβ-dependent and Aβ-independent mechanisms. There are still many doubts about the real pathogenesis of AD and the β-amyloid contribution to the onset of the disease. Aβ or Aβ oligomers or plaques are not solely responsible for the onset of the disease. More than 30 mutations responsible for FAD are localized in the APP gene; however, the "type London" APP mutations, causing only a slight increase in β-amyloid production, cause the onset of the pathology earlier than the "type Swedish" mutations, which induce a greater increase of the protein[229], suggesting that there are other mechanisms involved in the onset of AD. In fact, the Aβ-independent mechanisms are mediated via APP, intracellular fragments and PS1 via the cellular processes, such as inflammation, oxidative stress and Ca^{2+} dysregulation, implicated in AD pathogenesis[230]. Cdk5 may be influenced by or interact with both pathways, and its activation triggers DNA damage, cell cycle activation and neurodegeneration[231]. Non-Aβ factors such as Tau and ApoE also contribute to AD pathology[232]. All these pathways can lead to synaptic dysfunction, neurodegeneration and AD.

The senile plaques also do not seem to be an exclusive feature of Alzheimer's disease. They increase with age, even in healthy subjects, and the number of plaques in healthy controls is often comparable with the number found in age-matched affected individuals[233]. Moreover, β-amyloid is physiologically produced in healthy brains during neuronal activity and is necessary for synaptic plasticity and memory[234]. Furthermore, in the AD population, there is only a weak correlation between the number of senile plaques and the severity of the pathology. The cleavage of APP by γ-secretase produces some fragments called AICD (APP intracellular domain), which appear to play an important role in the onset of AD. In fact, it

is known that transgenic mice for AICD show tau phosphorylation and aggregation and decreased cell proliferation/survival, even in the absence of endogenous APP[235]. High levels of AICD may also play an important role in the pathology in human brains[236]. The challenges to the Amyloid Hypothesis of Alzheimer's Disease are sharply formulated[237]. There are several other competing hypotheses, such as the cholinergic hypothesis, the tau hypothesis, and the hypothesis that some other environmental risk factors, may contribute additional causes of the disease.

The cholinergic hypothesis proposed that AD is caused by cholinergic effects such as reduced synthesis of the neurotransmitter acetylcholine, or the initiation of large-scale aggregation of amyloid and neuroinflammation[238, 239]. Most currently available drug therapies are based on this hypothesis[240].

The genetic heritability of Alzheimer's disease reveals that most AD is caused by mutations in one of the genes that encoding APP and presenilins 1 and 2[241]. Most mutations in these genes increase Aβ42 production. Environmental and genetic risk factors, such as the ε4 allele of the apolipoprotein E (APOE)[242], increase the risk of the disease by threefold. Mutations in the TREM2 gene make the risk of developing Alzheimer's disease several times higher[243]. Other genes also appear to affect the risk, including *CASS4*, *CELF1*, *FERMT2*, *HLA-DRB5*, *INPP5D*, *MEF2C*, *NME8*, *PTK2B*, *SORL1*, *ZCWPW1*, *SlC24A4*, *CLU*, *PICALM*, *CR1*, *BIN1*, *MS4A*, *ABCA7*, *EPHA1*, and *CD2AP*[244].

The tau hypothesis postulates that tau protein abnormalities initiate the disease cascade as hyperphosphorylated tau forms neurofibrillary tangles, leading to the disintegration of microtubules in brain cells[245], which may result in dysfunction of the biological activity between neurons and later in the death of the cells.

Other hypotheses include such environmental risk factors as smoking and infection, and a neurovascular hypothesis has been proposed, suggesting that the blood-brain barrier is critical for brain Aβ homeostasis and regulates Aβ transport via the LRP receptor and RAGE, as mentioned before[246]. These findings point to new therapeutic targets for AD.

Aβ and inflammation

Aβ peptides are the major components of the senile plaques present in Alzheimer's disease. Recent studies have shown that the soluble assemblies of Aβ also stimulate neuronal dysfunction and may play a prominent role in stimulating the proinflammatory activation of primary microglia[247]. In the context of inflammation, compared to fibrillar assemblies, oligomer Aβ preparations induce greater or differential proinflammatory cytokine production by microglia and astrocytes *in vitro*[248]. Indeed, studies in primary glia demonstrate that the oligomer Aβ-induced increase in proinflammatory cytokines, such as nitric oxide, NO, TNFα and TNFβ secretion, occurs earlier and is greater than the increase induced by fibrillar assemblies of Aβ[249]. Thus, for different forms of Aβ, identifying their levels at different stages of AD, the inflammatory response they produce, and their underlying mechanisms (*eg*, receptor mediated) may provide critical information for therapeutic development.

Other aspects of biology of Aβ

In addition to the key role in the pathology of AD, Aβ generated in the brain and peripheral tissues also function in many other aspects of biology. Aβ has been shown to be a ligand with various receptors, as mentioned in the previous section. It can also be transported between tissues and across the blood-brain barrier by complex trafficking pathways[176] to destinations where it can induce and modulate proinflammatory activities in response to a variety of environmental stressors[250, 251]. Aβ also functions similarly to a group of biomolecules collectively known as "antimicrobial peptides" (AMPs) that function in the innate immune system. It inhibits the growth of eight of 12 clinically important pathogens screened[252] and acts as an anti-microbial peptide in several infection models including mice, *C elegans*, and cell culture models[253]. This new function stands in stark contrast to current models of Aβ-dependent pathology and will play significant roles in the development of future AD treatment strategies.

Therapeutic approaches for the treatment of Alzheimer's disease

Drugs approved by the FDA

To date, only a total of five drugs developed to improve the symptoms of Alzheimer's disease have been approved by the FDA. It is important to note that a new drug, Namzaric (donepezil and memantine)[254] was approved in 2014. The five drugs function by two different mechanisms. One is cholinesterase inhibition, which delays Alzheimer's disease by blocking hydrolysis of the critical neurotransmitter acetylcholine. This category of drugs includes donepezil (Aricept)[255, 256], approved in 1996; rivastigmine (Exelon)[255, 256], approved in 2000; and galantamine (Razadyne)[257], approved in 2001. The other one is memantine (Namenda)[258], approved in 2003, a non-competitive *N*-methyl-*D*-aspartate (NMDA) channel blocker that reduces the activity of the neurotransmitter glutamate, which plays an important role in learning and memory by binding to the NMDA receptor. Memantine can inhibit the prolonged influx of Ca^{2+} ions, particularly from extrasynaptic receptors, that forms the basis of neuronal excitotoxicity. It is an option for the management of patients with moderate to severe Alzheimer's disease. Namzaric is a combination of the two drugs to reduce the levels of both acetylcholine and glutamate (Table 2).

Novel therapeutic approaches for Alzheimer's disease

Researchers have identified several novel therapeutic approaches for Alzheimer's disease that focus on the reduction of amyloid oligomer levels. Methods that are currently under development include the inhibition of oligomerization using small molecule inhibitors, the neutralization of oligomeric species using immunotherapy, the overexpression of Aβ-degrading enzymes to control Aβ oligomer levels in the

Table 2. Summary of approved drugs for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
Donepezil (Aricept)	Cholinesterase inhibitor	Blocks acetylcholine neurotransmitter	[255, 256]
Rivastigmine (Exelon)	Cholinesterase inhibitor	Blocks acetylcholine neurotransmitter	[350]
Galantamine (Razadyne)	Cholinesterase inhibitor	Blocks acetylcholine neurotransmitter	[257]
Memantine (Namenda)	NMDA receptor antagonist	Blocks glutamate neurotransmitter and improves learning and memory	[258]
Donepezil and Memantine (Namzaric)	Cholinesterase inhibitor and NMDA receptor antagonist	Blocks acetylcholine and glutamate neurotransmitters and improves learning and memory	[254]

brain, catalytic Aβ antibodies to hydrolyze specific aggregates, β-sheet breakers to break existing β-sheet structures, Aβ blockers to block amyloid channels, and therapies directed against the tau protein to lead to the partial reversal of brain pathologies. All these approaches are in preclinical research stages, and their therapeutic efficiency remains unknown.

Small molecule inhibitors

These molecules (2-amino-4-chlorophenol, 4-aminophenol, 4-aminoanisole, 3,4-dihydroxybenzoic acid, 2-hydroxy-3-ethoxy benzoaldehyde) block Aβ oligomerization or fibrillization[259]. Fourteen naturally occurring polyphenolic compounds and polyphenol-containing black tea extracts inhibit the assembly of alpha-synuclein into multimeric oligomers, which are cytotoxic and share common structural elements with amyloid oligomers[260, 261]. Polyphenols derived from red wine prevent Aβ oligomerization and attenuate cognitive deterioration. The main phenolic component of olive oil, oleuropein, has been shown to possess antioxidant[262], anti-inflammatory[263] and hypolipidemic activities[264]. Small molecules (NQTrp, ClNQTrp, coumarin, furosemide, D737) that inhibit Aβ aggregation[265, 266] or remodel toxic soluble oligomers of Aβ[267] inhibit oligomer formation[268-274] (Table 3).

Secretase inhibitors and modulators

Since β-secretase and γ-secretase are responsible for the release of Aβ from the intracellular domain of APP, compounds that can partially inhibit the activity of either β- or γ-secretase have been extensively explored. β-Secretase inhibitors (*eg*, MK-8931, CTS21166) can block the first cleavage of APP inside the cell[275, 276]. A novel orally active β-secretase inhibitor, AZD3293, was tested in phase II/III clinical trials by Astra Zeneca and Eli Lilly[277]. γ-Secretase inhibitors can block the second cleavage of APP in the cell membrane and were expected to stop the subsequent formation of Aβ and its toxic fragments[278]. One γ-secretase inhibitor, semagacestat, was a candidate drug for a causal therapy against Alzheimer's disease, originally developed by Eli Lilly and Élan, but is unfortunately being stopped as there is no effect in phase III clinical trials[279]. An alpha-secretase agonist, EHT-0202[276], biases APP processing towards the non-amyloidogenic pathway. A new γ-secretase modulator, CHF5074, showed a longer survival time for treated animals[280]. Selective Aβ42-reducing agents (*eg*, tarenflurbil) modulate γ-secretase to decrease Aβ42 production in favor of shorter Aβ versions[281] (Table 4).

Immunotherapeutic approach

Immunotherapy stimulates the host immune system to recognize and attack Aβ or produces antibodies that enhance the clearance of Aβ oligomers or plaques to prevent plaque deposition. Active or passive Aβ immunization can prevent Aβ oligomerization, which is why antibodies to Aβ can be used to

Table 3. Summary of small molecule inhibitors of amyloid oligomers for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
2-Amino-4-chlorophenol	Blocks Aβ oligomerization and fibrillization	Blocks neurotoxicity	[259]
4-Aminophenol	Blocks Aβ oligomerization and fibrillization	Blocks neurotoxicity	[259]
4-Aminoanisole	Blocks Aβ oligomerization and fibrillization	Blocks neurotoxicity	[259]
3,4-Dihydroxybenzoic acid	Blocks Aβ oligomerization and fibrillization	Blocks neurotoxicity	[259]
2-Hydroxy-3-ethoxy benzoaldehyde	Blocks Aβ oligomerization and fibrillization	Blocks neurotoxicity	[259]
Resveratrol	Remodels soluble oligomers and amyloid fibrils into nontoxic species	Attenuates cognitive deterioration	[351]
NQTrp	Inhibits the fibrillization of amyloidogenic proteins	Reduces Aβ aggregation	[269, 270]
ClNQTrp	Inhibits the fibrillization of amyloidogenic proteins	Reduces Aβ aggregation	[269, 270]
Coumarin	Inhibits Aβ aggregation	Prevents cognitive decline	[266]
Furosemide	Inhibits Aβ oligomerization	Increases the life span	[273]
D737	Inhibits Aβ formation	Prevents toxicity and ROS accumulation	[274]

Table 4. Summary of secretase inhibitors and modulators for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
Semagacestat (LY450139)	γ-Secretase inhibitor	Reduces Aβ formation	[278]
CHF-5074	γ-Secretase modulators	Increases life span	[280]
MK-8931	β-Secretase inhibitor	Reduces Aβ levels	[275]
AZD3293	β-Secretase inhibitor	Reduces the production of Aβ	[277]
CTS21166	β-Secretase inhibitor	Reduce the amount of beta-amyloid	[276]
EHT-0202	α-Secretase agonist	Biases APP processing towards the non-amyloidogenic pathway	[276]
Tarenflurbil	Modulates β-secretase to reduce Aβ42 production	Potential treatment for Alzheimer's disease	[281]

decrease cerebral plaque levels. This decrease is accomplished by promoting microglial clearance and redistributing the peptide from the brain to the systemic circulation. Several epitopes of Aβ are exposed and available for antibody capture of the soluble peptides, while others are available for antibodies to bind with oligomers. One such Aβ vaccine is CAD106, currently in clinical trial[265], which induced efficacious Aβ antibody titers of different IgG subclasses mainly recognizing the Aβ3-6 epitope. The 20-amino-acid SDPM1 protein can bind to Aβ40 and Aβ42 tetramers and block subsequent Aβ amyloid accumulation. Aβ42 immunization leads to the clearance of amyloid plaques in patients with Alzheimer's disease but does not prevent progressive neurodegeneration[282]. A more recent study showed that programmed death 1 (PD-1) inhibitors, which are FDA-approved cancer drugs, may be effective in clearing Aβ plaques and improving cognitive performance in a mouse model of Alzheimer's disease[283]. Anti-Aβ antibodies (solanezumab, gantenerumab, crenezumab, IVIG), which can bind soluble Aβ and improve cognitive performance, are currently in clinical trials[165, 284, 285]. Solanezumab accommodates a large Aβ epitope (960 $Å^2$ buried interface over residues 16 to 26) that forms extensive contacts and hydrogen bonds to the antibody, largely via main-chain Aβ atoms and a deeply buried Phe19-Phe20 dipeptide core Solanezumab and crenezumab both share identity with the Aβ KLVFF epitope[286]. The human anti-Aβ monoclonal antibody, gantenerumab, binds Aβ plaques and targets the N-terminus and central portion of Aβ[287]. Intravenous immune globulin (IVIG) derived from human plasma contains IgGs that recognize conformational epitopes of Aβ fibrils and oligomers[288]. Intravenous immunoglobulin G[289] and 2E6[290] bind to soluble Aβ and reduce amyloid aggregation (Table 5).

Anti-aggregation agents

Anti-aggregation agents[291], such as apomorphine, can prevent Aβ peptides from aggregating or clear aggregates once they are formed[292]. The hormone melatonin may be effective against amyloid by interacting with dimers of the soluble Aβ peptide and inhibiting their aggregation[293-295]. The cannabinoid HU-210[296] has been shown to prevent Aβ-induced inflammation[297]. The endocannabinoids anandamide and noladin have also been shown to be neuro-protective against Aβ *in vitro*[298]. Apomorphine[299], melatonin[300], and tannic acid[301] can prevent Aβ aggregation. A number of small mol-

Table 5. Summary of immunotherapeutic approaches for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
CAD106	Aβ vaccine	Inhibits Aβ oligomerization and cytotoxicity	[352]
SDPM1	Aβ antibody	Blocks subsequent Aβ amyloid aggregation	[353]
PD-1 inhibitors	T-cell-mediated autoimmune meningoencephalitis	Clears Aβ plaques and improves cognitive performance	[354]
Gantenerumab	Humanized monoclonal antibody to Aβ	Binds to aggregated Aβ and reduces Aβ plaques in the brain	[284]
Solanezumab	Humanized monoclonal antibody to Aβ	Binds to soluble Aβ and reduces amyloid load via peripheral sink mechanism	[182]
Crenezumab	Humanized monoclonal antibody to Aβ	Inhibits aggregation and promotes disaggregation	[284]
IVIG	Human polyclonal anti-Aβ antibody	Binds to Aβ and reduces neurotoxicity	[285]
Intravenous immunoglobulin G	Human immunoglobulin preparation containing endogenous polyclonal antibodies to Aβ	Primarily binds to soluble Aβ and reduces amyloid load via peripheral sink mechanism	[289]
2E6	Heterodimer of immunoglobulin light chain variable domains	Hydrolyzes Aβ peptides	[290]

ecules extracted from traditional Chinese herbal medicine have been shown to be capable of inhibiting Aβ aggregation. Among them, LJW0F2 purified from the flowers of *Lonicera japonica Thunb* could inhibit Aβ42 aggregation and attenuate the cytotoxicity induced by Aβ42 aggregation[302]. Resveratrol, curcumin, EGb761, isoliquiritigenin, protocatechuic acid, atractylenolide III, chlorogenic acid, euphorbiafactor L3, euphorbiafactor L2, ganoderic acid D, and ganoderic acid DM extracts from Chinese herbal medicine can inhibit Aβ aggregation[303, 304]. A series of substituted bisphenol A derivatives function as Aβ aggregation inhibitors and can inhibit neurotoxicity and increase cell viability[305] (Table 6).

Aβ-degrading proteases (AβDPs)

Aβ can be degraded by a number of peptidases and proteinases, collectively known as Aβ-degrading proteases (AβDPs). Aβ-degrading proteases play an important role in Aβ degradation and may be a good target for the treatment of Alzheimer's disease. NEP has been reported to degrade Aβ oligomers that impair neuronal plasticity and cognitive function[306]. Several close homologues of NEP, NEP2[307] and human membrane metalloendopeptidase-like protein (hMMEL)[308], are also implicated in the degradation of Aβ[308]. Members of the M13 family of zinc metalloproteases, endothelin converting enzymes ECE1, ECE2, and ACE, are also known to be endogenous regulators of Aβ levels[142, 309]. The serine proteases plasmin, urokinase type and tissue type plasminogen activators (uPA and tPA, respectively) and acyl peptide hydrolase (APH) have been found to degrade Aβ both directly and indirectly[150, 310-312]. Several cysteine proteases, including cathepsin D[141], cathepsin B[282,313], BACE1[283, 291], and BACE2 [314] are also involved in Aβ degradation (Table 7).

Therapies directed against the tau protein

Neurofibrillary tangles (NFTs) caused by hyperphosphorylated tau are an important pathogenic factor in Alzheimer's disease, and the tau protein is therefore also an important biological target for innovative therapies. The inhibition of tau protein oligomerization and aggregation, tau phosphorylation, microtubule stabilization [epothilone D (BMS-241027), TPI-287][315], and the enhancement of tau degradation as well as tau immunotherapy (ACI-35[275]) are all potential strategies for Alzheimer's disease therapy. The tau protein hyperphosphorylation inhibitor LMTX can facilitate the clearance of tau from the brain and reduce Aβ aggregation and has reached phase three clinical trials[316]. The anti-tau AADvac1 vaccine is currently being investigated in phase II trials. AADvac1 has been reported to significantly improve neurobehavioral deficits and reduce neurofibrillary degeneration and mortality[317]. Moreover, glycogen synthase kinase 3 beta (GSK-3β) inhibitors, such as tideglusib and humulin R, can block the phosphorylation of tau protein and thus are potential drug targets for Alzheimer's disease[318] (Table 8).

Other blockers

A drug that is currently under investigation is liraglutide (Victoza), which is typically used as a diabetes drug. Treatment with Victoza improved object recognition and spatial recognition and resulted in cognitive benefits. Other histological benefits include a reduced inflammatory response and an increase in the number of young neurons in the dentate gyrus, where the Aβ level was also found to be significantly reduced[319]. The β-sheet breakers or blockers that are capable of binding Aβ consist of short synthetic peptides. They destabilize the β-sheet structure and inhibit the formation of Aβ oligomers or amyloids[320, 321]. Aβ oligomers can form calcium channels in membranes. Calcium conductance through these channels can be blocked by compounds MRS2481 and MRS2485, which destabilize the β-sheet structure and decrease Aβ-promoted neuronal toxicity[322]. Bexarotene might serve as another class of anti-Alzheimer compounds by efficiently preventing the cholesterol-dependent increase in calcium fluxes promoted by

Table 6. Summary of anti-aggregation agents for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
Apomorphine	Prevents Aβ aggregation	Reduces cellular toxicity	[299]
Hormone melatonin	Inhibits Aβ aggregation	Reverts amyloid deposition	[300]
Cannabinoid HU-210	Blocks microglial activation	Prevents Aβ-promoted inflammation	[296]
Tannic acid	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[301]
LJW0F2	Polysaccharide that blocks Aβ fibril formation	Reduces neurotoxicity	[302]
EGb761	Inhibits Aβ aggregation	Reduces neurotoxicity	[303]
Isoliquiritigenin	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Protocatechuic acid	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Atractylenolide III	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Chlorogenic acid	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Euphorbiafactor L3	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Euphorbiafactor L2	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Ganoderic acid D	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Ganoderic acid DM	Inhibits Aβ aggregation	Reduces neurotoxicity	[304]
Substituted bisphenol A derivatives	Inhibit Aβ aggregation	Inhibit neurotoxicity and increase cell viability	[305]

Table 7. Summary of β-degrading proteases (AβDPs) for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
NEP	Endogenous regulator of Aβ	Degrades Aβ oligomers	[306]
NEP2	Homologues of NEP	Degradation of Aβ	[307]
hMMEL	Homologues of NEP	Degradation of Aβ	[308]
ECE1	Endothelin converting enzyme	Endogenous regulator of Aβ	[142, 309]
ECE2	Endothelin converting enzyme	Endogenous regulator of Aβ	[142, 309]
ACE	Endothelin converting enzyme	Endogenous regulator of Aβ	[142, 309]
Plasmin	Serine protease	Degrades both monomeric and fibrillar forms of Aβ	[150, 355]
Acylpeptide hydrolase	Serine protease	Degrades secreted Aβ dimers and trimers	[356, 357]
Cathepsin D	Cysteine protease	Degradation of Aβ	[358]
BACE1	Cysteine protease	Degradation of Aβ	[291]
BACE2	Cysteine protease	Degradation of Aβ	[359]
Cathepsin B	Cysteine protease	Degradation of Aβ	[282]

Aβ in neural cells[323]. Voltage-gated calcium channel blockers, such as verapamil, diltiazem, isradipine and nimodipine, protect cultured neurons from Aβ-induced toxicity and thus could be potential candidates for treating Alzheimer's disease[324]. The 5-HT6 receptor antagonist idalopirdine, in combination with a cholinesterase inhibitor, may also increase cognitive function[325]. Huperzine A[326, 327], 2,2',4'-trihydroxychalcone (TDC)[328] and *bis*(7)-cognitin[329] exhibit neuroprotective effects. Agenin[330] and clioquinol[331] can inhibit Aβ deposition. Other inhibitors, such as the RAGE inhibitor azeliragon, the α7-nAChR inhibitor encenicline and the calcium antagonist nilvaldipine, can improve memory and could be further candidates for Alzheimer's disease therapeutics[332-334] (Table 9).

Amyloid dyes

The traditional method of identifying amyloid fibrils in tissue sections is by the use of amyloid-staining dyes. The first of these dyes was Congo red[335], whose staining is linked to the presence of the cross-β structure of fibrils. Other amyloid dyes include iodine-sulfuric acid[336], thioflavin T or S[337], crystal violet[338], methyl violet[339], BTA-1[340], chrysamine G[341], ANS (1-anilinonaphthalene-8-sulfonic acid)[342], *bis*ANS (4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonic acid)[342], Nile red[343], K114 ((*trans*, *trans*)-1-bromo-2,5-*bis* (4-hydroxystyryl) benzene)[344], FSB[345], curcumin[346], nanocurcumin[346], and others. The thioflavin T staining method is widely used to identify and classify amyloid proteins in tissues. Specific BTA-1 binding to Aβ plaques inhibits Aβ aggregation and cytotoxicity, making it a good drug candidate for Alzheimer's disease[340]. These amyloid dyes not only indicate the presence of mature amyloids but also function as a tool for dissecting their structure and the mechanism of amyloid formation. They bind selectively to Aβ in the human brain and blood vessels *in vitro*. They might thus lead to further compounds for the development of tracer agents for the *in vivo* diagnosis of Alzheimer's disease and of inhibitors of Aβ aggregation as a novel therapy for Alzheimer's disease (Table 10).

MicroRNAs

MicroRNAs (miRNAs) are a class of conserved endogenous small noncoding RNAs known to regulate the expression of complementary messenger RNAs involved in AD development[347]. MiRNAs in the brain play an important role in Aβ generation, targeting the mRNAs of APP, β-secretase and γ-secretase and altering Aβ expression. MiRNAs may provide a novel therapeutic approach to the treatment of AD while also providing new insights into the etiology of this neurological disorder[348]. A series of specific miRNAs can regulate APP

Table 8. Summary of novel therapeutic approaches directed against the tau protein for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
LMTX	Inhibitor of tau hyperphosphorylation	Facilitates clearance of tau from the brain and anti-Aβ aggregation	[316]
Epothilone D (BMS-241027)	Microtubule stabilizer	Increases BBB permeability and microtubule stability	[315]
TPI-287	Microtubule stabilizer	Increases BBB permeability and microtubule stability	[315]
AADvac1	Tau active vaccination	Improves neurobehavioral deficits, and reduces neurofibrillary degeneration and mortality	[317]
ACI-35	Anti-tau vaccine	Stimulates the immune system to produce antibodies which target the tau protein	[275]
Tideglusib	GSK-3β inhibitor	Blocks phosphorylation of tau protein	[318]
Humulin R	GSK-3β inhibitor	Blocks phosphorylation of tau protein	[318]

Table 9. Summary of other blockers for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
Liraglutide (Victoza)	A diabetes drug	Cognitive benefits, reduced inflammatory response and an increase of young neurons	[319]
β-Sheet breaker	Binds Aβ and destabilizes its structure	Inhibits oligomer or amyloid formation	[320, 321]
MRS2481	Small molecule blocker of Abeta channel	Protects neurons from Aβ induced toxicity	[322]
MRS2485	Small molecule blocker of Abeta channel	Protects neurons from Aβ induced toxicity	[322]
Bexarotene	Inhibits the binding of cholesterol to Aβ and prevents calcium-permeable amyloid pore formation	Anti-Alzheimer	[323]
Verapamil	Voltage-gated calcium channel blocker	Protects neurons from Aβ-induced toxicity	[324]
Diltiazem	Voltage-gated calcium channel blocker	Protects neurons from Aβ-induced toxicity	[324]
Isradipine	Voltage-gated calcium channel blocker	Protects neurons from Aβ-induced toxicity	[324]
Nimodipine	Voltage-gated calcium channel blocker	Protects neurons from Aβ-induced toxicity	[324]
Huperzine A	A novel lycopodium alkaloid	Neuroprotective effects	[326, 327]
Arctigenin	Inhibits Aβ production and promotes Aβ clearance	Ameliorates memory impairment	[330]
2,2',4'-trihydroxychalcone (TDC)	Represses beta-cleavage of APP and production of Aβ	Improves the memory impairment	[328]
Bis(7)-cognitin	Inhibition of AChE, NMDA receptor, nitric oxide synthase, and amyloid precursor protein/beta-amyloid cascade	Neuroprotective effects	[329]
Clioquinol	Inhibits metal-ion binding to Aβ	Inhibits Aβ deposition	[331]
Idalopirdine	5-HT_6 receptor antagonist	In combination with a cholinesterase inhibitor may increase the cognitive function	[325]
Azeliragon	RAGE inhibitor	Mediates transport of the Aβ peptide into the brain	[332]
Encenicline	α7-nAChR inhibitor	Restores memory function	[333]
Nilvaldipine	Calcium antagonist	Cognition improvement	[334]

expression and serve as an ideal target for AD therapeutic drug design. Both miR-107 and miR-29 may potentially target the mRNA of BACE1, which is the key enzyme responsible for generating Aβ protein from APP[349].

Conclusions

Aβ is the major component of senile plaques and participates in Alzheimer's disease progression through its neurotoxic effects. Identifying Aβ structures, biology, receptors and Aβ-based therapeutic approaches for the treatment of Alzheimer's disease therefore remains of paramount importance. In this review, we have addressed the different structures involved in Aβ accumulation and have discussed the current understanding of the biological function and neuro-

Table 10. Summary of amyloid dyes for the treatment of Alzheimer's disease.

Name	Targets	Effects	Ref
Congo red	Binds Aβ fibrils	Neuroprotective effects	[335]
Iodine-sulphuric acid	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[336]
Thioflavin-T	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[337]
Crystal violet	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[338]
Methyl violet	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[339]
BTA-1	Binds Aβ plaques	Inhibits Aβ aggregation and cytotoxicity	[340]
Chrysamine G	Binds Aβ deposits	Inhibits Aβ aggregation and cytotoxicity	[341]
ANS (1-anilinonaphthalene-8-sulfonic acid)	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[342]
*bis*ANS (4,4'-dianilino-1,1'-binaphthyl-5, 5'-disulfonic acid)	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[342]
Nile red	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	[343]
K114 ((*trans*,*trans*)-1-bromo-2,5-*bis* (4-hydroxystyryl)benzene)	Crosses the blood-brain barrier (BBB) and binds with amyloid plaques	Inhibits Aβ aggregation and inflammation	[344]
FSB	Binds Aβ deposits	Inhibits Aβ aggregation and cytotoxicity	[345]
Curcumin	Binds Aβ plaques	Detects Aβ plaques and as Aβ-specific antibody	[346]
Nanocurcumin	Binds Aβ plaques	Detects Aβ plaques and as Aβ-specific antibody	[346]

toxic role of Aβ and the potential receptors that interact with Aβ and mediate Aβ intake, clearance and metabolism. Identification of the key Aβ receptor under relevant physiological conditions and obtaining crystal structures of full-length Aβ in different states are critical for the development of new therapeutic agents.

Over the last decade, advances have been made in understanding the structures of Aβ peptide forms. Aβ peptide rapidly aggregates to form oligomers, protofibrils and fibrils that lead to the deposition of amyloid plaques. Different structural approaches, such as NMR spectroscopy, distance geometry, molecular dynamic techniques, and X-ray crystallography, have shown that the structural conversion of Aβ oligomers to fibrils involves the association of these loosely aggregated strands into α-helical and parallel β-sheet structures, as well as that the structural states transition quickly. Different signal transduction pathways are involved in Aβ expression, degradation, transport and clearance. The phosphorylation and activation of specific intracellular kinases represent common events in these signaling cascades, and these signaling molecules are potential targets for new Alzheimer's disease drugs.

Several therapeutic approaches for Alzheimer's disease target amyloid oligomers. Methods that are currently under development include the inhibition of Aβ oligomerization using small molecule inhibitors, the neutralization of oligomeric species using immunotherapy, the overexpression of Aβ-degrading enzymes in the brain, catalytic Aβ antibodies for hydrolyzing specific aggregates, β-sheet breakers for destabilizing existing β-sheet structure and Aβ blockers for blocking amyloid channels and thereby leading to the partial reversal of brain pathologies. The therapeutic targeting of microglia receptors implicated in the response to Aβ and their associated signaling pathways could reduce the inflammation associated with Alzheimer's disease. Tau protein inhibitors or vaccines and amyloid dyes that selectively bind Aβ and inhibit Aβ aggregation offer additional novel therapeutic approaches for the treatment of Alzheimer's disease.

We have summarized new progress in developing treatments targeting Aβ and its receptors. Existing Alzheimer's disease drugs only treat the symptoms of Alzheimer's disease; they do not decelerate or cure it. The last drug that was approved by the Food and Drug Administration for therapeutic Alzheimer's disease treatment was namzaric in 2014. In the last decade, several candidate drugs have failed to reach statistical significance in their primary outcomes. The drugs currently undergoing clinical trials are inhibitors of Aβ production and aggregation, Aβ antibodies and vaccines. Identification of the key physiological Aβ receptors and the determination of their crystal structures in complex with Aβ will play a critical role in mitigating Alzheimer's disease progression and symptoms.

Abbreviations

Amyloid beta (Aβ), amyloid precursor protein (APP), Aβ binding p75 neurotrophic receptor ($P75^{NTR}$), low-density lipoprotein receptor-related protein (LRP), cellular prion protein (PrP^{c}), metabotropic glutamate receptor (mGluR5), α subunit-containing nicotinic acetylcholine receptor (α7nAChR), *N*-methyl-*D*-aspartic acid receptor (NMDAR), β-adrenergic receptor (β-AR), erythropoietin-producing hepatocellular receptor (EphR), paired immunoglobulin-like receptor B (PirB).

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