Neurochemical Research (2019) 44:1289–1296
https://doi.org/10.1007/s11064-019-02756-x

ORIGINAL PAPER

Powering Amyloid Beta Degrading Enzymes: A Possible Therapy for Alzheimer's Disease

Nkumbu L. Sikanyika[1] · Helena C. Parkington[1] · A. Ian Smith[1] · Sanjaya Kuruppu[1]

Received: 12 October 2018 / Revised: 15 February 2019 / Accepted: 17 February 2019 / Published online: 26 February 2019

Abstract

The accumulation of amyloid beta (Aβ) in the brain is believed to play a central role in the development and progression of Alzheimer's disease. Revisions to the amyloid cascade hypothesis now acknowledge the dynamic equilibrium in which Aβ exists and the importance of enzymes involved in the production and breakdown of Aβ in maintaining healthy Aβ levels. However, while a wealth of pharmacological and immunological therapies are being generated to inhibit the Aβ-producing enzymes, β-site APP cleavage enzyme 1 and γ-secretase, the therapeutic potential of stimulating Aβ-degrading enzymes such as neprilysin, endothelin-converting enzyme-1 and insulin-degrading enzyme remains relatively unexplored. Recent evidence indicates that increasing Aβ degradation as opposed to inhibiting synthesis is a more effective strategy to prevent Aβ build-up. Therefore Aβ degrading enzymes have become valuable targets of therapy. In this review, we discuss the pathway of Aβ synthesis and clearance along with the opportunities they present for therapeutic intervention, the benefits of increasing the expression/activity of Aβ-degrading enzymes, and the untapped therapeutic potential of enzyme activation.

Keywords Alzheimer's disease · Amyloid beta · Amyloid beta degrading enzymes · Neprilysin · Endothelin-converting enzyme

Introduction: Alzheimer's Disease and Amyloid Beta

Alzheimer's disease (AD) is a neurogenerative disease characterised by the presence of various pathologies in the brain, in particular amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs). To date, there is no definitive answer as to the cause of AD, however current evidence suggests that many of the brain abnormalities seen in AD are initiated, or at least aggravated, by Aβ aggregation. Aβ oligomers have been shown to play a role in the formation of NFTs by promoting microtubule disassembly, tau misfolding and tau phosphorylation [1, 2]. Synapse loss associated with cognitive decline in AD is most pronounced in areas immediately surrounded by Aβ plaques [3, 4] and dystrophic neurites associated with Aβ plaques show greater signs of neurotoxicity than dystrophic neurites that are free in the neuropil [4]. Furthermore, mutations in genes that code for proteins involved in Aβ production such as the amyloid precursor protein (APP), presenillin 1 (PS1) and presenillin 2 (PS2) have been linked to AD pathogenesis [5, 6].

Correlations between Aβ accumulation and AD pathology led to the "amyloid cascade hypothesis" which proposes a link between an increase in brain Aβ and a decline in cognition [7, 8]. Over the years, revisions to the theory have been made to emphasise the key detail that Aβ exists in a dynamic equilibrium rather than an irreversible pathway. This equilibrium is maintained through a crucial balance between Aβ production and clearance. Disruption to this balance leads to increased Aβ deposition in the brain, eventually leading to the development of AD [7, 9] (Fig. 1). It has been shown in humans that increased Aβ deposition is the result of ineffective Aβ clearance as opposed to enhanced production [10]. This highlights the importance of targeting the Aβ clearance pathway in the treatment/management of AD.

Special Issue: In Honor of S.I. : Prof. Anthony J. Turner.

✉ Sanjaya Kuruppu
Sanjaya.Kuruppu@monash.edu

[1] Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia

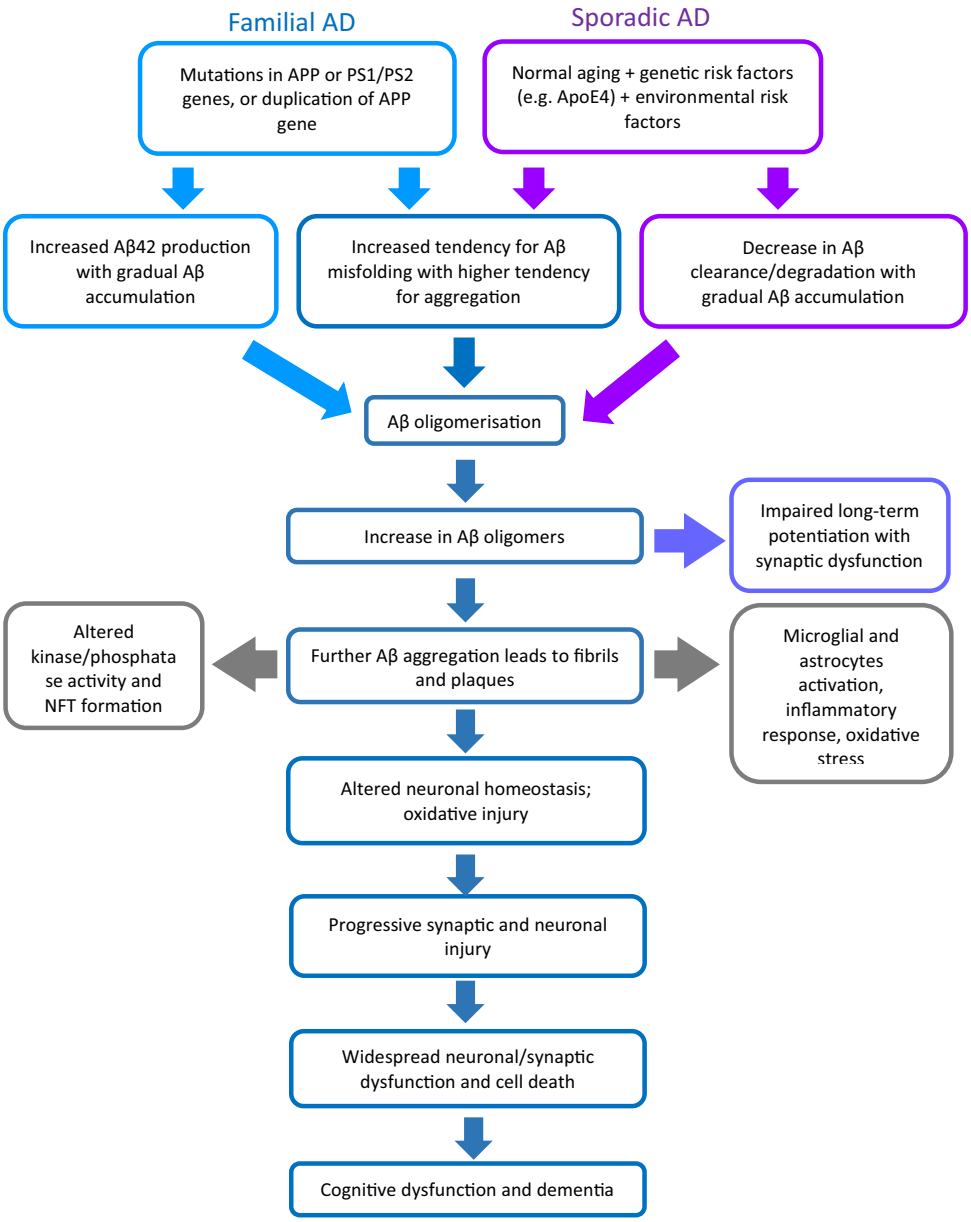


Fig. 1 The amyloid cascade hypothesis. In both familial and sporadic AD, genetic and environmental factors lead to increased Aβ production and decreased Aβ clearance. As Aβ gradually accumulates, oligomers form and consequently fibrils and plaques which disrupt inflammatory and immune responses. These disruptions alter homeostasis in neurons, leading to neuronal injury and dysfunction, which in turn leads to cell death, cognitive impairment and dementia

Targeting Aβ Pathways as Therapy for AD

Given the critical role of Aβ in the initiation of AD, it has been a target of AD therapy for decades. Therapeutic approaches targeting Aβ aim to either reduce synthesis or enhance clearance. Under normal physiological conditions, Aβ is produced by the sequential cleavage of its parent or precursor molecule, APP, by the proteases β-site APP cleavage enzyme 1 (BACE1) and γ-secretase [11, 12]. Once generated, Aβ has a variety of possible physiological roles including as a neuroprotective and positive modulator of neurotransmission, memory and synaptic plasticity [13], an antioxidant [14, 15], a regulator of cholesterol transport [16, 17], and a transcription factor [18, 19]. Aβ is then cleared via enzyme-mediated breakdown or through non-proteolytic pathways. The proteolytic degradation of Aβ is mediated by a wide range of proteases known as Aβ degrading enzymes (ADEs), of which there are almost 20 at present [20]. These include zinc-metalloproteases, serine, cysteine and aspartyl proteases. Alternatively, it may be removed by non-proteolytic clearance pathways, such as phagocytosis by microglia and astrocytes; interstitial fluid drainage through perivascular basement membranes; and transport across the blood vessel walls into circulation by the receptor low-density lipoprotein receptor-related protein 1 [21–23].

Reducing Aβ production has been the most widely explored therapeutic approach to reducing Aβ burden. This is typically achieved by targeting BACE1 or γ-secretase activities in order to inhibit Aβ production. Immunotherapeutic approaches have involved developing antibodies that hinder BACE1-mediated cleavage of APP. Certain anti-BACE1 antibodies bind to the active site of the enzyme to sterically prevent APP cleavage, while others regulate the catalytic domain of BACE1 by allosteric interactions [24]. Similarly, pharmacological agents have been developed to inhibit the activity of Aβ producing enzymes. BACE1 inhibitors have been thoroughly investigated, with many drug candidates such as lanabecestat (AZD-3293) and elenbecestat (E2609) currently progressing through clinical trials [25]. γ-secretase inhibitors have also been explored, with semagacestat being the first γ-secretase inhibitor to reach phase 3 clinical trials as a treatment for AD. However, further development was ceased after the drug was found to worsen the clinical condition of patients as well as increase the incidence of skin infection and skin cancer [26].

Certain non-steroidal anti-inflammatory drugs (NSAIDs) have also been tested as "γ-secretase modulators" following a reported correlation between long-term NSAID use and reduced incidences of AD by various epidemiological studies [27]. NSAIDs such as flurbiprofen and its enantiomer R-flurbiprofen, also known as Tarenflurbil, were classed as γ-secretase modulators after being found to influence γ-secretase activity to preferentially generate the more benign Aβ40 rather than the more fibrillogenic Aβ42 [28]. Tarenflurbil displayed the desired Aβ-lowering effects without the Cyclooxygenase (COX) inhibition exhibited by its prototype flurbiprofen and successfully progressed into clinical trials, however it was unable to prevent cognitive decline in patients with mild AD [29, 30]. Additionally, in-vivo studies in APP/PS1 mice found γ-secretase modulators to effectively reduce the production of new Aβ plaques but have no effect on existing plaques within the brain [31]. Such studies emphasise the need for drugs that can both break down existing Aβ plaque and prevent any future build-up, a goal that could be achieved by enhancing Aβ clearance pathways.

Aβ phagocytosis by microglia is one clearance pathway whose enhancement has been explored. "Aβ vaccines" have been investigated as a method of increasing Aβ phagocytosis to remove pre-existing build-up. Such vaccines contain either anti-Aβ antibodies that bind to endogenous Aβ to elicit passive immunity, or Aβ epitopes coupled with a carrier protein to bind B cells and elicit active immunity [24]. However, Aβ vaccine research experienced setbacks after a clinical trial for the Aβ vaccine AN1792 was halted when 6% of vaccinated patients developed aseptic meningoencephalitis [32]. An approach that could potentially be more useful but remains unexplored to date is stimulating the expression/activity of ADEs by drug like molecules.

Stimulating ADE Activity: A Viable Approach to Manipulating Aβ Levels

ADEs help to maintain Aβ equilibrium by degrading different pools of intracellular and extracellular Aβ within the brain [33, 34]. The decrease in protein expression and accumulation of oxidative stress associated with aging has been found to disrupt ADE expression and function [35]. This in turn contributes to Aβ accumulation potentially triggering AD, an observation that has been supported by many in-vitro and in-vivo studies. Most widely studied ADEs and a summary of evidence linking each enzyme to Aβ clearance is indicated in Table 1 along with the commonly known cleavage site(s) in Aβ(1–42). Variation in the cleavage site(s) reported for each enzyme could reflect the secondary degradation products identified and the different methods used for detection [36]. The subcellular localisation, substrates other than Aβ, and expression patterns of these enzymes within the human brain have been extensively described in previous reviews [37, 38] and hence will not be repeated here.

With much evidence demonstrating the benefit of increased ADE activity, strategies to enhance the ADE expression or activity could be a promising approach to manipulate Aβ levels. Indeed, the use of DNA based methods such as gene therapy to increase ADE expression in-vivo has been explored and yielded promising results. Gene delivery of NEP and ECE-1 to APP transgenic mice and APP/PS1 mice respectively was found to reduce Aβ burden in the brain [53]. However, DNA based techniques are difficult to translate into a clinical setting given the ethical concerns surrounding the safety to the individual and the risk of unintentional (or deliberate) modification of the human germline [54].

Stimulating the activity of ADEs through pharmacological agents would be a more feasible alternative with the same physiological outcome, and molecules capable of this have already been identified. Two IDE-activating compounds, named la1 and la2, have been shown to increase IDE-mediated Aβ degradation by 700 and 400% respectively [55]. Similarly, several compounds have been found to stimulate NEP activity including polyphenols found in green tea extract [56], the hormone humanin [57] and the tryptophan metabolite kynurenic acid [58]. However, the effect of these molecules on the breakdown of Aβ are yet to be determined, nor have they undergone any detailed pharmacological and biochemical characterisation. At present, there are no ADE stimulating drugs available in the clinic.

Most recently, we reported the discovery and pharmacological characterisation of a venom-derived peptide, which increases the activity of both NEP and ECE-1 [59]. Venoms

Table 1 Widely known Aβ degrading enzymes and their cleavage site(s) along Aβ(1–42)

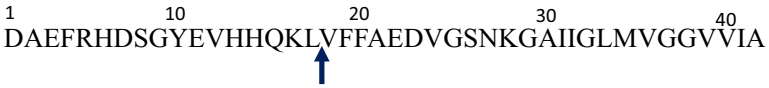
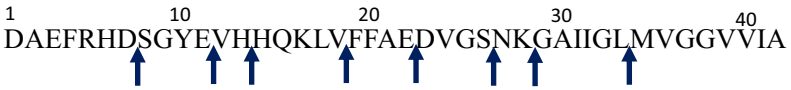
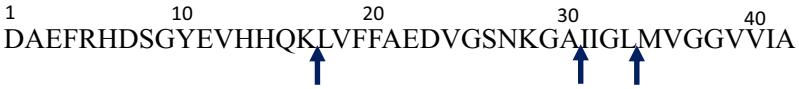
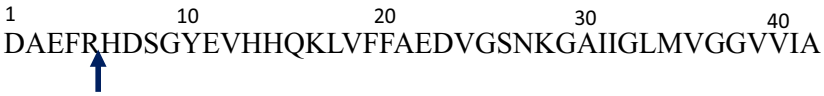
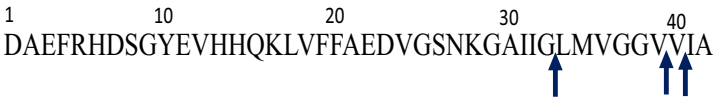
ADEs	Evidence linking to Aβ clearance	Cleavage sites along Aβ(1–42)*
NEP-1	• Use of the NEP inhibitor thiorphan caused an increase in Aβ42 deposition in rats [33] • The use of NEP inhibitors caused increases in Aβ and consequently plaque deposition in rat and mouse brain [39, 40] • Studies conducted in APP transgenic mice revealed NEP overexpression greatly reduced plaque build-up [41] and improved cognition in certain tests [42]	
NEP-2	• NEP-2 knockout mice show a 1.5-fold increase in total Aβ species in both brain stem and hippocampus [43] • Increase in Aβ build up was more pronounced in NEP-2 knockout mice cross bred with APP transgenic mice	
ECE-1	• Use of the dual ECE/NEP inhibitor phosphoramidon caused greater Aβ deposition in cultured neuronal cells than the selective NEP inhibitor thiorphan [33] • The brains of ECE-1 knockout mice shown elevated Aβ levels in comparison to controls [44]	
IDE	• British precursor protein 2 (BRI 2) overexpression leads to increased IDE levels and consequently a reduction in Aβ levels in APP/PS1 mouse model of AD and in HeLa cells [45] • IDE −/− mice displayed increased Aβ levels in the brain, and Aβ breakdown in brain membrane fractions and neuronal cultures was reduced by more than 50% [46]	
ACE	• $ACE^{10/10}$ mice (which overexpress ACE in myelomonocytes) crossed with AD + mice (i.e. APP/PS1 transgenic mice) produced offspring that displayed lower levels of Aβ42 as well as decreased Aβ plaque load in the brain than the AD + parent mice [47] • The vasodilator and ACE inhibitor drug ramipril caused greater Aβ deposition in AD + $ACE^{10/10}$ mice than the ACE-independent vasodilator hydralazine [47]	
MMP-2 and −9	• The use of an MMP inhibitor increased Aβ levels in the brain interstitial fluid of APP transgenic mice [48] • The brains of MMP-2 and −9 KO mice showed higher Aβ levels than wild-type controls [48]	
Plasmin	• Levels of plasminogen activator inhibitor-1 (PAI-1), a protein that inhibits plasmin production, correlates with Aβ levels in the brain whereas plasmin activity has an inverse correlation with Aβ levels [49] • PAI-1 KO mice expressing human APP and PSEN1 showed significantly reduced Aβ levels [49] • The use of PAI-1 inhibitors causes a decrease in Aβ levels in the brain and plasma of transgenic mice expressing human Aβ [50]	

Table 1 (continued)

ADEs	Evidence linking to Aβ clearance	Cleavage sites along Aβ(1–42)*
Cathepsin B	• Effectively cleaves Aβ(1–42) producing C-terminally truncated peptides [51] • Increasing the expression of Cathepsin B in hAPP mice reduces existing Aβ deposits [51] • Inactivating Cathepsin B expression increases Aβ deposition [51]	
Acyl peptide hydrolase	• Cell culture based studies indicate acyl peptide hydrolase preferentially degrade dimeric and trimeric forms of Aβ • Increased expression of the enzyme in human brain regions (temporal cortex) showing large amounts of Aβ. This suggests enzyme expression is elevated in response to increased Aβ levels [52]	

*Arrows indicate the cleavage site while numbers above the sequence indicate the amino acid number

have indeed been used in drug development with the ACE inhibitor captopril perhaps being the best-known example [60]. In addition, exenatide for managing diabetes [61] and prialt for pain [62] are more recent examples of venom derived drugs. The peptide, which we called K49-P1-20, was derived from the venom of *Bothrops asper* [59]. It corresponds to the first 20 amino acids of *B. asper* myotoxin II found in this venom. K49-P1-20 increased the activity of both NEP and ECE-1 by 1605 ± 58 and 1563 ± 23% of control respectively at a concentration of 100 ng/μL [59]. The activation concentration 50 (AC50) of K49-P1-20 against NEP and ECE-1 are 1.33 ± 0.12 and 1.92 ± 0.07 μM respectively indicating increased selectivity for NEP [59]. Initial studies also indicate that K49-P1-20 directly interacts with both enzymes thus inducing a conformational change [59]. Prior incubation of enzyme with K49-P1-20 significantly enhanced the breakdown of both synthetic Aβ40 and soluble endogenous Aβ42 in cerebrospinal fluid of AD patients taken post-mortem [59]. K49-P1-20 also significantly increased the activity of IDE and ACE-2, making it the first known activator of multiple ADEs and a potential drug lead for AD [59].

In addition to Aβ, ADEs can cleave a range of other substrates [37, 38]. Therefore it appears logical to assume that increasing the activity of these can lead to a multitude of responses effecting multiple organ systems including the endocrine, nervous and cardiovascular systems. However, critical for a physiological response is a corresponding increase in the substrate level. Therefore in the absence of an increase in substrate level, enhanced enzyme activity alone is unlikely to have an effect.

The Untapped Therapeutic Potential of Enzyme Activation

While ADE activators represent a viable therapeutic approach to treating AD, our understanding of the mechanism(s) behind enzyme activation is poor. This is a stark contrast to our knowledge on enzyme inhibition and the wealth of studies describing enzyme inhibitors and their mechanisms of action [63, 64]. Many diseases are exacerbated by either increased or decreased enzyme activity, however our approaches to treatments for these diseases are very different. Conditions that are worsened by increased enzyme activity are often treated with enzyme inhibitors such as ACE inhibitors for hypertension [65] and statins for hypercholesterolemia [66]. However, conditions that are caused by insufficient enzyme activity, such as phenylketonuria, are typically managed by limiting substrate availability rather than with administration of an enzyme activator [67]. As a result, enzyme inhibition has been well researched and is a common mode of action of currently marketed drugs, whereas relatively little is known about the possibilities of enzyme activation. The apparent lack of knowledge around enzyme activation reflects the poor availability of enzyme activators, particularly those targeting amyloid beta degrading enzymes. In addition, much attention has focused on DNA based techniques aimed at enhancing enzyme expression as opposed to stimulating their activity.

This information, taken together, provides strong evidence for the use of molecules such as K49-P1-20 to help improve our understanding of the mechanism(s) behind enzyme activation and unlock the secrets of proteolysis. Further research could also enable the rational design of analogues with more suitable pharmacodynamic/pharmacokinetic properties and thus result in a new drug class for the treatment of AD.

Future Directions

AD is a debilitating, neurodegenerative disease with no effective treatment at present. The accumulation of Aβ in the brain is believed to exacerbate and/or instigate various AD-related pathologies including NFT formation, synapse loss and dystrophic neurites. As a result, the pathways for Aβ synthesis and Aβ degradation have become targets of therapy in the quest to find an effective treatment for AD.

Pharmacological and immunological therapies that target the Aβ-producing enzymes BACE1 and γ-secretase have thus far only delivered somewhat mixed results, however it is clear that such therapies would only prevent further Aβ accumulation and not clear any pre-established build-up. Increasing ADE-mediated Aβ degradation could be a more effective approach given that it would remove both new and pre-existing pools of Aβ, thus holding the potential to prevent or even reverse the progression of AD.

A large number of anti Aβ therapies including antibody based therapies have failed to reach the clinic. This has led to a re-evaluation of the amyloid hypothesis. However, it is also believed that anti Aβ therapies tested were administered at a late stage thus offering little hope for positive clinical outcomes. The pathophysiological process underlying AD is thought to begin decades before the onset of mild cognitive impairment and diagnosis [68]. This raises the question of developing appropriate methods for the early identification of patients most at risk of developing AD. To this end, a recent study has identified a panel of blood based biomarkers which can accurately predict brain Aβ status at individual level thereby allowing broader population based screening [69]. This could also allow potential novel anti Aβ approaches such as K49-P1-20 to be targeted to individuals most at risk.

Using pharmacological agents as opposed to gene therapy to increase ADE-mediated Aβ degradation, also presents as a more viable strategy from an efficiency, economical and ethical standpoint. Furthermore, enzyme activators such as K49-P1-20 can be used to increase our knowledge on the mechanism(s) of enzyme activation and potentially usher in a new class of drugs to help fight AD and other diseases.

Acknowledgements This work was supported by the Virginia Wilke and John Farrell Postgraduate Research Scholarship, the Yulgilbar Alzheimer's Research Program and Monash University.

References

1. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1:a006189
2. Zempel H, Thies E, Mandelkow E, Mandelkow EM (2010) Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J Neurosci 30:11938–11950
3. Shankar GM, Walsh DM (2009) Alzheimer's disease: synaptic dysfunction and Abeta. Mol Neurodegener 4:48
4. Tsai J, Grutzendler J, Duff K, Gan WB (2004) Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. Nat Neurosci 7:1181–1183
5. Muller U, Winter P, Graeber MB (2013) A presenilin 1 mutation in the first case of Alzheimer's disease. Lancet Neurol 12:129–130
6. Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81:741–766
7. Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297:353–356
8. Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256:184–185
9. Nalivaeva NN, Belyaev ND, Zhuravin IA, Turner AJ (2012) The Alzheimer's amyloid-degrading peptidase, neprilysin: can we control it? Int J Alzheimers Dis 2012:383796
10. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ (2010) Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science 330:1774
11. Chow VW, Mattson MP, Wong PC, Gleichmann M (2010) An overview of APP processing enzymes and products. Neuromol Med 12:1–12
12. Haass C, Kaether C, Thinakaran G, Sisodia S (2012) Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med 2:a006270
13. Puzzo D, Privitera L, Leznik E, Fa M, Staniszewski A, Palmeri A, Arancio O (2008) Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. J Neurosci 28:14537–14545
14. Baruch-Suchodolsky R, Fischer B (2009) Abeta40, either soluble or aggregated, is a remarkably potent antioxidant in cell-free oxidative systems. Biochemistry 48:4354–4370
15. Guglielmotto M, Aragno M, Autelli R, Giliberto L, Novo E, Colombatto S, Danni O, Parola M, Smith MA, Perry G, Tamagno E, Tabaton M (2009) The up-regulation of BACE1 mediated by hypoxia and ischemic injury: role of oxidative stress and HIF1alpha. J Neurochem 108:1045–1056
16. Igbavboa U, Sun GY, Weisman GA, He Y, Wood WG (2009) Amyloid beta-protein stimulates trafficking of cholesterol and caveolin-1 from the plasma membrane to the Golgi complex in mouse primary astrocytes. Neuroscience 162:328–338
17. Yao ZX, Papadopoulos V (2002) Function of beta-amyloid in cholesterol transport: a lead to neurotoxicity. FASEB J 16:1677–1679
18. Bailey JA, Maloney B, Ge YW, Lahiri DK (2011) Functional activity of the novel Alzheimer's amyloid beta-peptide interacting domain (AbetaID) in the APP and BACE1 promoter sequences and implications in activating apoptotic genes and in amyloidogenesis. Gene 488:13–22
19. Maloney B, Lahiri DK (2011) The Alzheimer's amyloid beta-peptide (Abeta) binds a specific DNA Abeta-interacting domain (AbetaID) in the APP, BACE1, and APOE promoters in a sequence-specific manner: characterizing a new regulatory motif. Gene 488:1–12
20. Nalivaeva NN, Beckett C, Belyaev ND, Turner AJ (2012) Are amyloid-degrading enzymes viable therapeutic targets in Alzheimer's disease? J Neurochem 120(Suppl 1):167–185
21. Bates KA, Verdile G, Li QX, Ames D, Hudson P, Masters CL, Martins RN (2009) Clearance mechanisms of Alzheimer's amyloid-beta peptide: implications for therapeutic design and diagnostic tests. Mol Psychiatry 14:469–486

22. Deane R, Wu Z, Sagare A, Davis J, Du Yan S, Hamm K, Xu F, Parisi M, LaRue B, Hu HW, Spijkers P, Guo H, Song X, Lenting PJ, Van Nostrand WE, Zlokovic BV (2004) LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. Neuron 43:333–344
23. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV (2000) Clearance of Alzheimer's amyloid-ss(1–40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. J Clin Investig 106:1489–1499
24. Atwal JK, Chen Y, Chiu C, Mortensen DL, Meilandt WJ, Liu Y, Heise CE, Hoyte K, Luk W, Lu Y, Peng K, Wu P, Rouge L, Zhang Y, Lazarus RA, Scearce-Levie K, Wang W, Wu Y, Tessier-Lavigne M, Watts RJ (2011) A therapeutic antibody targeting BACE1 inhibits amyloid-beta production in vivo. Sci Transl Med 3:84ra43
25. Yan R (2016) Stepping closer to treating Alzheimer's disease patients with BACE1 inhibitor drugs. Transl Neurodegener 5:13
26. Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, Kieburtz K, He F, Sun X, Thomas RG, Aisen PS, Alzheimer's Disease Cooperative Study Steering C, Siemers E, Sethuraman G, Mohs R, Semagacestat Study G (2013) A phase 3 trial of semagacestat for treatment of Alzheimer's disease. N Engl J Med 369:341–350
27. Vlad SC, Miller DR, Kowall NW, Felson DT (2008) Protective effects of NSAIDs on the development of Alzheimer disease. Neurology 70:1672–1677
28. Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC, Ozols VV, Jessing KW, Zavitz KH, Koo EH, Golde TE (2003) NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 in vivo. J Clin Investig 112:440–449
29. Green RC, Schneider LS, Amato DA, Beelen AP, Wilcock G, Swabb EA, Zavitz KH, Tarenflurbil Phase 3 Study G (2009) Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. JAMA 302:2557–2564
30. Szekely CA, Green RC, Breitner JC, Ostbye T, Beiser AS, Corrada MM, Dodge HH, Ganguli M, Kawas CH, Kuller LH, Psaty BM, Resnick SM, Wolf PA, Zonderman AB, Welsh-Bohmer KA, Zandi PP (2008) No advantage of A beta 42-lowering NSAIDs for prevention of Alzheimer dementia in six pooled cohort studies. Neurology 70:2291–2298
31. Garcia-Alloza M, Subramanian M, Thyssen D, Borrelli LA, Fauq A, Das P, Golde TE, Hyman BT, Bacskai BJ (2009) Existing plaques and neuritic abnormalities in APP:PS1 mice are not affected by administration of the gamma-secretase inhibitor LY-411575. Mol Neurodegener 4:19
32. Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C (2003) Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. Neurology 61:46–54
33. Eckman EA, Reed DK, Eckman CB (2001) Degradation of the Alzheimer's amyloid beta peptide by endothelin-converting enzyme. J Biol Chem 276:24540–24548
34. Pacheco-Quinto J, Herdt A, Eckman CB, Eckman EA (2013) Endothelin-converting enzymes and related metalloproteases in Alzheimer's disease. J Alzheimers Dis 33(Suppl 1):S101–S110
35. Nalivaeva NN, Turner AJ (2017) Role of ageing and oxidative stress in regulation of Amyloid-degrading enzymes and development of neurodegeneration. Curr Aging Sci 10:32–40
36. Turner AJ, Nalivaeva NN (2013) Peptide degradation (Neprilysin and other regulatory peptidases). In: Kastin AJ (ed) Handbook of biologically active peptides. Elsevier, San Diego, pp 1757–1764
37. Miners JS, Barua N, Kehoe PG, Gill S, Love S (2011) Abeta-degrading enzymes: potential for treatment of Alzheimer disease. J Neuropathol Exp Neurol 70:944–959
38. Saido T, Leissring MA (2012) Proteolytic degradation of amyloid beta-protein. Cold Spring Harb Perspect Med 2:a006379
39. Dolev I, Michaelson DM (2004) A nontransgenic mouse model shows inducible amyloid-beta (Abeta) peptide deposition and elucidates the role of apolipoprotein E in the amyloid cascade. Proc Natl Acad Sci USA 101:13909–13914
40. Nisemblat Y, Belinson H, Dolev I, Michaelson DM (2008) Activation of the amyloid cascade by intracerebroventricular injection of the protease inhibitor phosphoramidon. Neuro-degener Dis 5:166–169
41. Marr RA, Rockenstein E, Mukherjee A, Kindy MS, Hersh LB, Gage FH, Verma IM, Masliah E (2003) Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. J Neurosci 23:1992–1996
42. Marr RA, Spencer BJ (2010) NEP-like endopeptidases and Alzheimer's disease. Curr Alzheimer Res 7:223–229
43. Hafez D, Huang JY, Huynh AM, Valtierra S, Rockenstein E, Bruno AM, Lu B, DesGroseillers L, Masliah E, Marr RA (2011) Neprilysin-2 is an important beta-amyloid degrading enzyme. Am J Pathol 178:306–312
44. Eckman EA, Watson M, Marlow L, Sambamurti K, Eckman CB (2003) Alzheimer's disease beta-amyloid peptide is increased in mice deficient in endothelin-converting enzyme. J Biol Chem 278:2081–2084
45. Kilger E, Buehler A, Woelfing H, Kumar S, Kaeser SA, Nagarathinam A, Walter J, Jucker M, Coomaraswamy J (2011) BRI2 protein regulates beta-amyloid degradation by increasing levels of secreted insulin-degrading enzyme (IDE). J Biol Chem 286:37446–37457
46. Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ, Guenette S (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci USA 100:4162–4167
47. Bernstein KE, Koronyo Y, Salumbides BC, Sheyn J, Pelissier L, Lopes DH, Shah KH, Bernstein EA, Fuchs DT, Yu JJ, Pham M, Black KL, Shen XZ, Fuchs S, Koronyo-Hamaoui M (2014) Angiotensin-converting enzyme overexpression in myelomonocytes prevents Alzheimer's-like cognitive decline. J Clin Investig 124:1000–1012
48. Yin KJ, Cirrito JR, Yan P, Hu X, Xiao Q, Pan X, Bateman R, Song H, Hsu FF, Turk J, Xu J, Hsu CY, Mills JC, Holtzman DM, Lee JM (2006) Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid-beta peptide catabolism. J Neurosci 26:10939–10948
49. Liu RM, van Groen T, Katre A, Cao D, Kadisha I, Ballinger C, Wang L, Carroll SL, Li L (2011) Knockout of plasminogen activator inhibitor 1 gene reduces amyloid beta peptide burden in a mouse model of Alzheimer's disease. Neurobiol Aging 32:1079–1089
50. Jacobsen JS, Comery TA, Martone RL, Elokdah H, Crandall DL, Oganesian A, Aschmies S, Kirksey Y, Gonzales C, Xu J, Zhou H, Atchison K, Wagner E, Zaleska MM, Das I, Arias RL, Bard J, Riddell D, Gardell SJ, Abou-Gharbia M, Robichaud A, Magolda R, Vlasuk GP, Bjornsson T, Reinhart PH, Pangalos MN (2008) Enhanced clearance of Abeta in brain by sustaining the plasmin proteolysis cascade. Proc Natl Acad Sci USA 105:8754–8759
51. Mueller-Steiner S, Zhou Y, Arai H, Roberson ED, Sun B, Chen J, Wang X, Yu G, Esposito L, Mucke L, Gan L (2006) Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. Neuron 51:703–714

52. Yamin R, Zhao C, O'Connor PB, McKee AC, Abraham CR (2009) Acyl peptide hydrolase degrades monomeric and oligomeric amyloid-beta peptide. Mol Neurodegener 4:33
53. Hemming ML, Patterson M, Reske-Nielsen C, Lin L, Isacson O, Selkoe DJ (2007) Reducing amyloid plaque burden via ex vivo gene delivery of an Abeta-degrading protease: a novel therapeutic approach to Alzheimer disease. PLoS Med 4:e262
54. Jin X, Yang YD, Li YM (2008) Gene therapy: regulations, ethics and its practicalities in liver disease. World J Gastroenterol 14:2303–2307
55. Cabrol C, Huzarska MA, Dinolfo C, Rodriguez MC, Reinstatler L, Ni J, Yeh LA, Cuny GD, Stein RL, Selkoe DJ, Leissring MA (2009) Small-molecule activators of insulin-degrading enzyme discovered through high-throughput compound screening. PLoS ONE 4:e5274
56. Ayoub S, Melzig MF (2006) Induction of neutral endopeptidase (NEP) activity of SK-N-SH cells by natural compounds from green tea. J Pharm Pharmacol 58:495–501
57. Niikura T, Sidahmed E, Hirata-Fukae C, Aisen PS, Matsuoka Y (2011) A humanin derivative reduces amyloid beta accumulation and ameliorates memory deficit in triple transgenic mice. PLoS ONE 6:e16259
58. Klein C, Patte-Mensah C, Taleb O, Bourguignon JJ, Schmitt M, Bihel F, Maitre M, Mensah-Nyagan AG (2013) The neuroprotector kynurenic acid increases neuronal cell survival through neprilysin induction. Neuropharmacology 70:254–260
59. Smith AI, Rajapakse NW, Kleifeld O, Lomonte B, Sikanyika NL, Spicer AJ, Hodgson WC, Conroy PJ, Small DH, Kaye DM (2016) N-terminal domain of *Bothrops asper* myotoxin II enhances the activity of endothelin converting enzyme-1 and neprilysin. Sci Rep 6:22413
60. Camargo AC, Ianzer D, Guerreiro JR, Serrano SM (2012) Bradykinin-potentiating peptides: beyond captopril. Toxicon 59:516–523
61. Furman BL (2012) The development of Byetta (exenatide) from the venom of the Gila monster as an anti-diabetic agent. Toxicon 59:464–471
62. Hamad MK, He K, Abdulrazeq HF, Mustafa AM, Luceri R, Kamal N, Ali M, Nakhla J, Herzallah MM, Mammis A (2018) Potential uses of isolated toxin peptides in neuropathic pain relief: a literature review. World Neurosurg 113:333–347 e335
63. Dannhardt G, Kiefer W (2001) Cyclooxygenase inhibitors–current status and future prospects. Eur J Med Chem 36:109–126
64. McFarlane SI, Kumar A, Sowers JR (2003) Mechanisms by which angiotensin-converting enzyme inhibitors prevent diabetes and cardiovascular disease. Am J Cardiol 91:30H–37H
65. Sweitzer NK (2003) What is an angiotensin converting enzyme inhibitor? Circulation 108:e16–e18
66. Istvan ES, Deisenhofer J (2001) Structural mechanism for statin inhibition of HMG-CoA reductase. Science 292:1160–1164
67. van Spronsen FJ (2010) Phenylketonuria: a 21st century perspective. Nat Rev Endocrinol 6:509–514
68. Mehta D, Jackson R, Paul G, Shi J, Sabbagh M (2017) Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010–2015. Expert Opin Investig Drugs 26:735–739
69. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Dore V, Fowler C, Li QX, Martins R, Rowe C, Tomita T, Matsuzaki K, Ishii K, Ishii K, Arahata Y, Iwamoto S, Ito K, Tanaka K, Masters CL, Yanagisawa K (2018) High performance plasma amyloid-beta biomarkers for Alzheimer's disease. Nature 554:249–254