Are N- and C-terminally truncated Aβ species key pathological triggers in Alzheimer’s disease?

Published, Papers in Press, August 24, 2018, DOI 10.1074/jbc.R118.003999

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Edited by Paul E. Fraser

The histopathology of Alzheimer’s disease (AD) is characterized by neuronal loss, neurofibrillary tangles, and senile plaque formation. The latter results from an exacerbated production of amyloid-β (Aβ) peptides that are produced by sequential cleavages of amyloid precursor protein (APP) by β- and γ-secretases. The amyloid cascade hypothesis proposes a key role for the full-length Aβ precursor protein (APP) by γ-secretase in AD etiology, in which soluble Aβ oligomers lead to neurotoxicity, tau hyperphosphorylation, aggregation, and, ultimately, cognitive defects. However, following this postulate, during the last decade, several clinical approaches aimed at decreasing full-length Aβ42 production or neutralizing it by immunotherapy have failed to reduce or even stabilize AD-related decline. Thus, the Aβ peptide (Aβ40/42)-centric hypothesis is probably a simplified view of a much more complex situation involving a multiplicity of APP fragments and Aβ catabolites. Indeed, biochemical analyses of AD brain deposits and fluids have unraveled an Aβ peptidome consisting of additional Aβ-related species. Such Aβ catabolites could be due to either primary enzymatic cleavages of βAPP or secondary processing of Aβ itself by exopeptidases. Here, we review the diversity of N- and C-terminally truncated Aβ peptides and their biosynthesis and outline their potential function/toxicity. We also highlight their potential as new pharmaceutical targets and biomarkers.

A defining characteristic of Alzheimer’s disease pathology is the presence of extraneuronal plaques composed of aggregated Aβ species (Aβ). Aβ terminology usually refers mainly to a mix of canonical 40/42 amino acid peptides excised by endoproteolysis of a type I transmembrane protein called β-amyloid precursor protein (βAPP) through the sequential action of two enzymes: β-site APP-cleaving enzyme (BACE1) and γ-secretase (Fig. 1A) (1). Processing of βAPP by these two enzymatic activities also generates an intracellular fragment, APP intracellular domain (AICD), that behaves as a transcription factor (2, 3). Once produced, Aβ peptides are secreted and, upon various triggers that could be of genetic or environmental natures, accumulate and yield oligomeric aggregates. These oligomeric structures can transiently remain soluble or ultimately fibrillize and seed to form one of the main histological stigmata of AD pathology, senile plaques. However, the number of plaques is not clearly associated with disease progression and severity. Recent studies suggest that soluble, oligomeric forms of Aβ have an important role in neurotoxicity and memory loss (4). Thus, these oligomeric species of Aβ have been shown to cause synaptic dysfunction, to disrupt long-term potentiation (LTP) (5, 6), and to affect behavior in transgenic mice (7, 8).

The amyloid cascade hypothesis is strongly supported by genetic facts. Thus, all mutations responsible for early onset and aggressive forms of AD share as common denominators a modulation of total Aβ load, modification of Aβ42 over the Aβ40 ratio, or generate Aβ species prone to aggregation (9, 10). Thus, the amyloid cascade hypothesis is at the center of gravity of AD pathology. However, the vast majority of clinical trials centered on Aβ by either blocking their production or neutralizing them once produced have failed. It is thus a challenge to reconcile genetic grounds with clinical failures. It could be envisioned that the physiological function of full-length Aβ could have been underestimated. Furthermore, the contribution of Aβ-derived fragments generated by secondary cleavages or additional APP-derived fragments distinct from Aβ could have been underestimated. Thus, other fragments issued from βAPP processing, such as the membrane-tethered fragment, C99, or βCTF, could very well contribute to pathological dysfunctions (11–13). Also, one can consider the fact that several aspects of physiological βAPP processing have still to be delineated. This is exemplified by recent data showing that besides classical secretases, novel proteolytic actors recently came on stage. For example, recent works unmasked a novel cleavage site on βAPP (14, 15), named Eta (η) cleavage site (14), which gives rise to a subset of new fragments (Aηα, Aηβ, and ηCTF) (Fig. 2). The enzyme responsible for βAPP cleavage at the η site has been identified as the matrix metalloproteinase MT5–MMP (15, 16).

This work was supported by LABEX (Excellence Laboratory, Program Investment for the Future), DISTALZ (Development of Innovative Strategies for a Transdisciplinary approach to Alzheimer’s disease), the Hospital University Federation (FHU) OncoAge, and the Fondation Plan Alzheimer. The authors declare that they have no conflicts of interest with the contents of this article.

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2 The abbreviations used are: Aβ, amyloid-β, AD, Alzheimer’s disease; AICD, APP intracellular domain; Aβ, amyloid precursor protein; BACE1, βAPP-cleaving enzyme; CA, cornu ammonis-1; CSF, cerebrospinal fluid; DPPIV, dipeptidyl peptidase 4; FAD, familial Alzheimer’s disease; QC, glutaminyl cyclase; LTP, long-term potentiation; MCI, mild cognitive impairment; MMP, matrix metalloproteinase; MT5, membrane type-5; NSAID, non-steroidal anti-inflammatory drug; CTF, C-terminal fragment.

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J. Biol. Chem. (2018) 293(40) 15419–15428 15419

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In this Minireview, we will focus on the N- and C-terminally truncated /\beta\text{-amyloid} peptides that are produced either by primary cleavages taking place on /\beta\text{APP} or yielded by secondary cleavages occurring on /\beta\text{A} full-length /\beta40 or /\beta42. We will describe here several N- and C-terminally truncated /\beta\text{A} peptides, their production, biophysical properties in terms of aggregation, neuronal toxicity, as well as their putative impact on Alzheimer’s disease progression. We will also describe the
potential of N- and C-terminally truncated Aβ peptides as biomarkers as well as pharmaceutical targets.

**Truncated fragments resulting from primary cleavages on βAPP**

Aside the well-known conventional full-length Aβ40 and Aβ42 peptides, several shorter Aβ peptides have been identified. Some of them are directly generated by primary cleavages taking place on βAPP. Thus, BACE1-mediated cleavage of βAPP at the β′ site (17, 18) combined with γ-secretase cleavage yields Aβ(11–40/42), whereas α/γ-secretase cleavages generate Aβ(17–40/42) peptides (Fig. 1B).

It is interesting to note that FAD-linked mutations occurring on βAPP or on the γ-secretase–associated proteins presenilin-1 and presenilin-2 (19) usually result in either an increase in total Aβ load or, alternatively, a selective increase in Aβ42 and thereby Aβ42/Aβ40, and consistently lead to early-onset AD (20). Interestingly, Ancolio et al. (21) showed that cells overexpressing a BAPP bearing the V715M mutation indeed behave differently because they secrete less Aβ40, whereas levels of Aβ42 remained unchanged. More strikingly, they described a 7-fold increase in the recovery of Aβ(11/17–42) with no change in Aβ(11/17–40) (21). These data suggest the potential toxic effect of 11/17-X fragments in AD pathology. This conclusion is supported by both anatomical, biophysical, and clinical grounds. Thus, these shorter fragments have been described in senile plaques as well as in AD and Down syndrome brains. These fragments are increased by FAD mutations on PS1 as well (22). Finally, both cleavage fragments could indicate a pathophysiological condition because expressions of the enzymes responsible for their production are regulated during AD. Moreover, Aβ(11–X) and Aβ(17–X), have been both detected in cerebrospinal fluids in patients suffering from mild cognitive impairment (MCI), i.e. at very early stages of the disease (23). Although levels of Aβ(11–X) in CSF of MCI patients seem to be lowered, Aβ(17–X) tends to be expressed at higher levels than in controls. Moreover, Aβ40 appeared to be higher in the cerebrospinal fluid of patients with Alzheimer’s disease than in patients suffering from other dementias (24).

de Strooper and co-workers (25) documented the fact that slight alterations of the Aβ40/42 ratio could lead to drastic modifications in toxic potential. Ancolio et al. (21) support this view and further indicate that the ratio of Aβ-truncated fragments could also account for early onset cases of AD even when the total Aβ load is reduced.

**Truncated fragments resulting from secondary cleavages on Aβ peptides**

Truncated Aβ species could just represent by-products of Aβ degradation. However, these could also harbor their own spectrum of physiological and/or (according to their concentration) toxic functions. In the latter case, they could be seen as biotransformation derivatives of Aβ. Thus, this questions the potential weight of truncated Aβ species in AD pathology. As a corollary, they could represent a new set of early diagnostic markers, and thus, enzymatic activities implicated in their formation could be seen as potential therapeutic targets.

**MINIREVIEW: Truncated Aβ species in Alzheimer's disease**

**C-terminally truncated Aβ species**

Aβ38, Aβ37, and Aβ39

Although a plethora of articles have addressed the biology of Aβ40 and Aβ42, the C-terminally truncated Aβ peptides (Fig. 3) have received much less consideration. However, in addition to Aβ40 and Aβ42, several Aβ shorter species truncated in their C-terminal moiety, among them Aβ37, Aβ38, and Aβ39, have been identified in blood plasma samples and human cerebrospinal fluids of patients suffering from AD (26). Aβ38 was even found to be the second prominent Aβ form after Aβ40 in quantity. These peptides may be produced according to different pathways. The stepwise release of the GVV and VIA tripeptides generates Aβ37 and Aβ39 from Aβ40 and Aβ42, respectively. Moreover, Aβ37 can be produced by another pathway after release of the GVVIA peptide from the Aβ42 sequence (27). Production of Aβ38 is influenced by nonsteroidal anti-inflammatory drugs (NSAIDs). Although NSAID treatments tend to globally decrease Aβ42 production, these compounds induce a slight shift of βAPP cleavage leading to an increase of Aβ38 formation (28), and this appears to occur independently of their primary target, Cox2. Aβ38 production has been shown to be increased by a subset of γ-secretase modulators (29).

Immunohistological studies of Aβ37 and Aβ39 C-terminally truncated peptides in AD brains and transgenic mouse models have revealed that both peptides were found to accumulate in meningeal and parenchymal vessels in the brains of familial AD cases as well as in sporadic AD (30). The pattern of deposition differs between AD brains and transgenic mouse models. In sporadic or familial AD brains, the C-terminally truncated peptides appeared to be aggregated in plaques, but in transgenic mouse models, the presence of truncated peptides in plaques was more variable.

Interestingly, expression of shorter species of Aβ, from Aβ37 to Aβ40, does not elicit toxicity in Drosophila and even appeared to attenuate Aβ42 toxicity (31). Such results suggest that treatments regulating βAPP processing by favoring an increase in Aβ37, Aβ38, or Aβ39 production could be somehow beneficial and seen as an Aβ42-related inactivating pathway.

**C-terminally truncated Aβ34 variant**

It has been demonstrated that Aβ34 derived from hydrolysis of Aβ by BACE1 (32, 33). Aβ34 is increased in cells overex-
pressing both human βAPP and human BACE1, and it is interesting to note that a γ-secretase inhibitor treatment impairs Aβ34 levels suggesting that Aβ34 production results from a secondary cleavage that occurs after Aβ release by γ-secretase (34).

BACE1 inhibition decreases Aβ34 in CSF (35). However, in vitro experiments have also pointed out that Aβ34 could be produced by a secondary cleavage step on Aβ40 due to matrix metalloproteases (MMP2 and MMP9) (36). These MMPs could also degrade Aβ34 into shorter species (Aβ30 and Aβ16).

Aβ34 physiology and the impact on AD progression have not been deeply assessed. This may be due to a lack of tools to directly monitor the peptide and assess its pathophysiological influence. Caillaud et al. (37) characterized and developed an AβX-34 – directed specific polyclonal antibody recognizing the C-terminal part of AβX-34. They demonstrated that AβX-34 peptides are present in 3xTgAD mice brains as well as in AD patient’s brains. More recently, it has been shown by immuno-fluorescence and immunohistological studies in three different mouse models that Aβ34-like immunoreactivity appears as a punctate diffuse pattern and does not label the center of senile plaques (38). Moreover, a similar histological pattern was found in the brains of AD patients, and several studies have shown that Aβ34 is an abundant species in CSF of AD patients (26, 38, 39). Thus, BACE1 contributes to Aβ production but is also involved in its secondary cleavage as well. Whether a disruption in the balance governing and the equilibrium between Aβ and Aβ34 underlies part of AD pathology or whether BACE1 could be seen as a beneficial enzyme contributing to Aβ catabolism remains to be established. At first sight, the age-related increase in BACE1 activity and the associated elevation of Aβ34 argue in favor of the first hypothesis. However, Caillaud et al. (37) showed that Aβ34 could display a protective phenotype in HEK293 cells. It is possible that the Aβ34-associated protective phenotype could be abolished when its aggregation occurs as has been documented for Aβ (40). In this context, it is noteworthy that, as is the case for Aβ (2, 41), Aβ34 undergoes degradation by nephrilysin, a peptidase, the activity and expression of which are reduced during aging. Thus, both age-related augmentation of BACE1 (42) and reduction of nephrilysin expressions and activities could very well account for augmentation of Aβ34 levels, aggregation, and pathogenic phenotype.

### Aβ24

C-terminally truncated Aβ24 (Aβ1–24)) is a peptide produced upon secondary cleavage of full-length Aβ after activation of glial cells. Such a peptide has not yet been described in the brains of AD patients. However, it has been shown that intracranial injection of synthetic Aβ24 in WT mice impairs full-length Aβ42 clearance through the blood–brain barrier and promotes Aβ42 aggregation via its seeding properties. Moreover, the synthetic Aβ24 peptide tends to promote Aβ42 aggregation, whereas the Aβ24 peptide itself presents a low-aggregation propensity (43). The exact nature of the enzyme responsible for Aβ24 production still remains unknown. However, it is interesting to notice that a cleavage between a valine and a glycine, as is the case for Aβ24 generation, is not a usual signature of endopeptidases or exopeptidases. Noteworthy, some matrix metalloproteinas (MMP-9 or MMP-2), which are regulated by microglial activation, appeared to be involved in Aβ degradation, generating C-terminally truncated fragments such as Aβ23, Aβ30, or Aβ34 (36). Their involvement in Aβ24 production still awaits firm demonstration.

### N-terminally truncated Aβ species

**Aβ2-X**

The amino-truncated β-amyloid peptide Aβ(2–42) has been detected in a detergent-soluble fraction of AD brains (44) as well as in the CSF of sporadic and familial AD patients. Does this Aβ peptide have a physiological or a pathological function or is it only an intermediate form for another cleavage giving rise to a shorter peptide? Even if Aβ(2–42) has been described in CSF and brains of AD patients, very little is known about this Aβ species and its biological properties. However, an important aspect concerned the fact that, unlike the case for canonical Aβ species (45), Aβ(2–42) production remains poorly affected by presenilin-1 deficiency in neurons (44), although γ-secretase cleavage is obviously necessary for releasing this fragment. This could be explained by a selective involvement of presenilin-2 in Aβ42 formation. This would imply that PS1 and PS2 occur in distinct cellular compartments, one of which is permissive for Aβ(2–42) generation. Indeed, recent works show that PS1 and PS2 occur in distinct cellular compartments (46, 47). Alternatively, one cannot rule out the possibility that a PS1-independent activity(ies) (48–52) could account for at least part of Aβ(2–42) production.

**Aβ3-X and pE3-XAβ**

Another pyroglutamate-modified Aβ that begins with the glutamate in position 3 of Aβ (pE3-XAβ) has been described. Several lines of anatomical clues suggest a potential key role of this Aβ species in AD pathology. Thus, pE3-XAβ has been shown to be present in quantities similar to full-length Aβ in senile plaques (53) but also in diffuse plaques (54) and in the vascular wall (55). Noticeably, pE3-XAβ is also present in Down’s syndrome-affected brains (56). pE3-42Aβ appeared to be a dominant isoform in the hippocampus and cortex in patients with AD (57).

pE3-XAβ fragments can trigger hippocampal neuronal loss, microglial activation, and astroglisis and impair long-term potentiation in transgenic animals expressing human pE3-XAβ (58). This toxicity appears to be accounted for by the ability of pE3-XAβ to seed Aβ and promote its deposition (59).

pE3-XAβ peptide formation is a two-step process involving an N-terminal truncation releasing the first two Aβ residues Asp–Ala followed by an enzymatic cyclization of the glutamyl in position 3 (Fig. 4). Studies aimed at deciphering the enzymes responsible for Aβ3-X formation and pyroglutamate conversion have highlighted several candidates. Sevalle et al. (60) delineated the contribution of aminopeptidase A (APA) to the truncation of full-length Aβ leading to the 3-X species. Thus, by means of two distinct selective inhibitors, APA overexpression and APA-expressing membranes, it was demonstrated that the initial step consisting of the release of the aspartyl 1 residue was elicited by APA. This was consistent with the APA’s affinity for
acridic residues (61). APA seems not to be the only exopeptidase responsible for N-terminal truncations of Aβ and exposition of glutamate at position 3. Implication of a member of the dipeptidyl peptidase family enzymes (DPP) has been previously suggested (62). Recently, in vitro experiments using MALDI-TOF MS applications have pointed out the possible implication of DPP IV in Aβ3-X formation (63). Interestingly, Aβ40 peptide appeared to be the more prone to DPP IV truncation compared with Aβ42.

After removal of the two first residues by APA and/or the dipeptidyl aminopeptidase activities, the glutamate residue at position 3 is converted into pyroglutamate forming a peptide more resistant to exopeptidase attack. Several anatomical, pharmacological, and genetic evidences indicated that the enzyme responsible for Aβ3-X cyclization was an acyltransferase named glutaminyl cyclase (QC) (64–66). First, QC is unevenly distributed in brain and is up-regulated in AD-affected brains (67). Second, QC protein and mRNA expressions colocalize with pE3- Aβ in human temporal and entorhinal cortices and, more importantly, correlate better with cognitive alterations assessed by mini-mental state examination than the unmodified Aβ peptides (40). Third, in animal models, QC overexpression triggers behavioral deficits, whereas conversely, QC depletion rescues defects observed in an AD-transgenic model (68). Fourth, pharmacological blockade of endogenous QC by selective inhibitors reduces the pE3-XAβ load in mouse and Drosophila AD models and reduces plaques, astrogliosis, and cognitive alterations in AD mouse models (64). It should be added that calcium homeostasis dysregulation, which is commonly observed in cellular AD models, increases QC mRNA expression and activity in neuron-like differentiated SK-N-SH (69). It should be noted that β’ cleavage mediated by BACE1 (see above and Fig. 1B) generates a glutamyl residue that can undergo cyclization. Whether GC is involved in pE11-XAβ remains to be established.

N-truncated Aβ4-X

N-truncated Aβ(4–42) was one of the first Aβ-truncated species being reported (70). This truncated form, which starts with a phenylalanine at position 4, was found to be highly abundant in AD brains, aged controls, and vascular dementia (57, 71). It has been demonstrated that this peptide rapidly formed stable aggregates (72). Aβ4-X species concentrate in the core of the plaques in several AD mouse models (38). In vitro toxicity assays showed that Aβ(4–42) is as toxic as pE3Aβ and Aβ42 (72), but Aβ(4–40) was less toxic compared with Aβ(4–42). In terms of the kinetics of appearance, Aβ4-X variants seemed to precede pE3-XAβ accumulation in the 5×FAD transgenic mouse models (73). In vivo studies have indicated that intraventricular injections of Aβ(4–42) in WT mice tend to affect working memory as assessed with a Y maze test (72). Little is known about the catalytic events responsible for Aβ4-X production. One can envision an exopeptidasic release of either glutamate at position 3 that would occur before its cyclization or removal of the pE3 residues once formed. Based on theoretical grounds, two types of peptidases could indeed perform these cleavages. On the one hand, free glutamate residues could be released by acidic peptidase such as aminopeptidase A that is already involved in Asp-1 removal (see above and Ref. 60). On the other hand, there exists two types of pyroglutamyl peptidases I and II that are specialized in the attack of pE residues (61). In vitro studies with fluorimetric substrates show that the enzyme cleaves tripeptides where the third Xaa could be indeed interchanged but only TRH natural substrate fulfills the requirement and is cleaved (74). Pyroglutamyl peptidase I only requires a pE residue without clear requirement for residues in the P2 or P3 position. Assessment of its involvement in Aβ4-X genesis is still pending.

Biophysical properties of truncated peptides

Amyloid peptide aggregation is a hallmark of AD pathology. However, precise mechanisms leading to seed formation and
accelerated aggregation during the disease progression are still under investigation. Infusion of brain extracts derived from AD transgenic mouse model in WT rodent brains is not per se sufficient to trigger Aβ aggregation (75). Also, aged synthetic Aβ40 and Aβ42 did not trigger deposits in APP23 transgenic mice (75). This suggests first that in physiological conditions, an efficient clearance mechanism occurs that keeps the Aβ concentration below its threshold of aggregation. This also indicates that cellular, genetic, or environmental cofactors may govern seeding formation and its acceleration in pathological conditions (75). Interestingly, this increased capability to induce full-length Aβ seeding has been described for several truncated Aβ variants such as Aβ24 (43) and pE3Aβ (76).

Glutamate cyclization results in the loss of a negative charge that contributes to the hydrophobicity, resistance to catabolism (77), and aggregation propensity of N-terminally truncated Aβ species pE11–Aβ and pE3–Aβ. pE3–42Aβ influences folding of full-length unmodified Aβ (76). Moreover, pEÅ peptides are known to seed the aggregation of other Aβ fragments. It has been suggested that the C-terminal part of Aβ42 was the locus of interaction with pE3–Aβ (78). Aside pE–Aβ variants, Aβ(4–42) has also a propensity to form very stable aggregates (72). Fast aggregation properties of such fragments have been described in vitro (59, 79) as well as in vivo in a Drosophila model (80).

Increased aggregation propensity and related toxicity have been described also for pyrog glutaminylated forms of ADn and ABri, two peptides signing Familial Danish dementia and Familial British dementia, respectively (79, 81, 82).

Neuronal loss and synaptic loss in the hippocampus have been related to the early stages of AD. Several studies have pointed out the effects of truncated Aβ peptides on cellular toxicity. Some Aβ species protect against Aβ42 toxicity, whereas others appeared to be more aggressive than full-length Aβ peptides. As described above, Aβ34 protects cells overexpressing βAPP WT or bearing Swedish mutation from caspase-3-related cell death (37), and therefore, in physiological conditions it could be considered as a beneficial isoform. This protective phenotype could be hampered by Aβ34 aggregation. On the contrary, pE3–Aβ behaves like a noxious peptide. It has a high capacity to induce lipid peroxidation and to influence membrane permeabilization in primary cultured neurons (83). Toxicity of pE3–42Aβ, assayed on rat cultured hippocampal neurons, appeared to be increased compared with the toxicity of full-length Aβ (77). More recently, development of transgenic mice expressing pE3Aβ peptides has helped to demonstrate that such truncated forms are implicated in neuronal loss (58). A Drosophila model has been characterized where pE3–42Aβ peptide is expressed in neurons. Interestingly, the life span of transgenic flies was affected by pE3–42Aβ. Moreover, expression of the pyrog glutaminylated peptide induced behavioral dysfunctions, and toxicity was observed by a disorganization of the eye structure (80). Finally, pE3–42Aβ has recently been shown to induce synaptic plasticity impairment by different mechanisms than Aβ42 and independently of a co-oligomerization process (84). Precise mechanisms involved in pE3–42Aβ toxicity have still to be addressed.

**Truncated Aβ species in animal models**

Several truncated and modified Aβ species have been found in AD murine models. C-terminally truncated Aβ37 and Aβ39 are widely expressed in the vasculature of human sporadic and familial AD patients (30). The same study also addressed Aβ37 and Aβ39 expressions in several transgenic AD mouse models (APP/PS1ΔE9, 5xFAD, PDAPP, APP23, 3xTgAD, and APP/PS1KI). However, expression patterns appeared to be drastically distinct. In mice, both C-terminally truncated β-amloid peptides were found in plaques, but vascular expression was almost absent in all the mouse models tested. Aside from Aβ40 and Aβ42, several other Aβ peptides were found in the 5xFAD mouse model. The more abundant truncated peptide detected by MS was Aβ38, followed by Aβ(4–42), pE3–42Aβ, and Aβ39. Aβ(4–40), Aβ(5–42), and Aβ37 were also present but to a lesser extent (30).

A transgenic mouse model expressing the N-truncated Aβ(4–42) peptide in the brain has been engineered (Tg(4–42)) to investigate the effect of a chronic exposure of this toxic peptide that appears particularly abundant in human brain (57, 70). This transgenic mouse model has been shown to express Aβ(4–42) in the CA1 region of the hippocampus. However, such expression decreased with aging because of a massive neuronal loss in the region (72) associated with working memory dysfunction. Moreover, neurodegeneration was supported by early astroglialosis and microglial activation at only 2 months of age. The Tg(4–42) mouse model showed spatial memory deficits starting at 5 months of age and being severely impaired at 6 months of age (85). In this model, Aβ(4–42) hippocampal expression correlates with a significant neuron loss in the CA1 layer of the hippocampus (85).

Another transgenic mouse model, with a glutamine instead of the glutamate at position 3 of the Aβ peptide, has been generated to examine the effect of cyclization on pathology development (86). The TBA42 mice that do not express βAPP present a very rapid onset of symptoms, accumulate pE3–Aβ, and harbor microglial activation and impaired LTP (58). These mice showed an age-dependent neuronal loss in the CA1 region of the hippocampus. TBA42 mice were then crossed to 5xFAD to engineer the FAD42 mouse model, which at 6 months of age showed an aggravated phenotype compared with the 5xFAD mouse model (87). This set of data corroborates the view of a pathological influence of pE3–Aβ species.

**Aβ truncated species as biomarkers**

Almost all truncated Aβ species, yielded by primary cleavages of βAPP or secondary cleavages directly on Aβ, have been detected in the CSFs and therefore could represent an interest as putative early biomarkers. Thirteen C-terminally truncated species have been detected in CSF (88, 89). Quantitation of several Aβ species reveals, for example, that Aβ1–38 decreases in AD fluids compared with controls CSF (39). It appears that the pattern of truncated Aβ could help differentiate between some neurological disorders. For example, Aβ2–42 levels in cerebrospinal fluids are decreased in AD, although they are unchanged in fronto-temporal dementia (90). Aβ(11–X) and Aβ(17–X) peptides are of interest as new
biomarkers for MCI detection because they have been identified in cerebrospinal fluids in patients with very early MCI (23).

Pharmaceutical strategies

As discussed in this Minireview, truncated amyloid species and pyroglutamate Aβ are very toxic forms of Aβ, and thus, they correspond to potential therapeutic targets. One of the advantages of a therapeutic strategy aimed at abolishing truncated Aβ species-related pathology relies on the fact that such an approach will not interfere with any physiological function of soluble full-length Aβ (40, 60, 91). Accordingly, two main classical strategies could be envisioned that concern immunotherapy aimed at neutralizing truncated or modified Aβ or inhibitors of enzymes implicated in truncation and cyclization.

A specific antibody, targeting Aβ42-X variants (NT4X-167), has been developed and characterized in transgenic mouse models as well as sporadic and FAD patients (73). Although the NT4X-167 antibody reacts with senile plaques in 5xFAD mouse models, a different pattern was found in AD brains where staining was observed in blood vessels as in Down’s syndrome cases (92). Considering also Aβ(4–42) as a target for a therapeutic strategy appeared relevant because passive immunization with NT4X antibody decreases neuronal loss in CA1, rescues spatial memory deficits in Tg(4–42) mouse model, and reduced amyloid plaques in 5xFAD mice (85). A chronic passive immunization against pE3 in the APPsw/PS1ΔE9 transgenic mouse model also triggers beneficial effects on plaque deposition, cerebral amyloid angiopathy, as well as glosis (93). Another pharmaceutical strategy relies on the effect of QC inhibition to prevent formation of pyroglutaminylated Aβ species. Several QC inhibitors are actually in development (64, 94). Obviously, a pre-requisite of these pharmacological approaches remains the firm identification of enzymes responsible for fragment formation. As stated above, in some cases, this identification is still awaited. Additional problems to overcome could be related to the lack of exclusive specificity displayed by these peptidases (61). Thus, close examination of the potential side-effects linked to proteolysis of additional substrates, a key feature that was initially underestimated when γ-secretase inhibitors were designed and envisioned as therapeutic probes, will be a priori and redhibitory requirement.

Conclusion

The monitoring of full-length Aβ peptides (Aβ(1–40) and Aβ(1–42)) as biomarkers of AD pathology has to be deeply reconsidered with respect to the fact that many additional Aβ-related species are generated and recovered in biological fluids. Secondary cleavages onto a canonical Aβ peptide sequence have only been considered for a while as a clearance paradigm, aimed at depleting Aβ(1–40) and Aβ(1–42) and generating biologically inert bypass products. More likely, it appears that it gives rise to new players with potential pathological properties. Many Aβ truncated peptides that aggregate or favor seed aggregation yield variable oligomer profiles. Whether this represents a pathogenic signature accounting for specific differences observed in variable settings and the progression of AD in patients has to be envisioned.

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