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Mini-Review

How an *APP* gene mutation protects against Alzheimer's disease?

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ABSTRACT

In familial Alzheimer's disease (AD), many pathogenic mutations have been reported for amyloid-β precursor protein (*APP*) and presenilin 1 and 2 genes. However, only one protective mutation has been reported for the *APP* gene, in Icelanders. Although APP metabolism is closely related to intracellular trafficking of APP and APP-cleaving enzymes, the mechanism of the causative gene mutations remains largely unclear. The Icelandic A673T mutation of the *APP* gene protects against AD, whereas the A673V mutation at the same amino acid position is pathogenic. APP A673T markedly decreases generation of CTFβ/C99, which is produced in the amyloidogenic cleavage of APP by β-site APP-cleaving enzyme 1 (BACE1). This CTFβ/C99 is further cleaved by γ-secretase to generate neurotoxic amyloid-β 1-40 ($Aβ^{1-40}$) and $Aβ^{1-42}$ peptides. Because BACE1 activity is increased in intracellular acidic compartments, it has been suggested that APP A673T may escape from this BACE1-dependent amyloidogenic pathway. However, we propose a novel hypothesis that BACE1 preferentially cleaves APP A673T at the β′- rather than the β-site, generating an amino-terminally truncated $Aβ^{11-XX}$. This alternative β′-site cleavage of APP decreases the generation of $Aβ^{1-XX}$ forms, which is how the A673T mutation of APP functions protectively against AD. Similarly, APP A673V is preferentially cleaved at the β-site rather than the β′-site. This increases the generation of CTFβ/C99 and the subsequent $Aβ^{1-XX}$ forms, compared with wild-type APP, and is the mechanism whereby APP A673V functions pathogenically. The previous suggestion that the molecular and cellular mechanisms underpinning the protective A673T mutation of APP occurred *via* decreased amyloidogenic processing of APP has been controversial. Our novel finding that the amino acid sequence within the Aβ region alters a property of APP cleavage by BACE1 is likely, rather than the conventional explanation that an APP mutation induces intracellular trafficking of APP into an amyloidogenic pathway.

KEYWORDS: Alzheimer's disease, BACE1, APP, intracellular trafficking

ABBREVIATIONS

AD, Alzheimer's disease; APP, amyloid β-precursor protein; Aβ, amyloid β; BACE1, β-site APP-cleaving enzyme-1; CTF, C-terminal fragment of APP truncated at the primary cleavage site.

INTRODUCTION

There are many mutations on the amyloid β-precursor protein (*APP*) and presenilin (*PSEN*)-1 and *PSEN*2 genes in familial Alzheimer's disease (FAD) [1]. Almost all of these are pathogenic for AD through the increased generation of amyloid-β (Aβ) peptides or the facilitated production of Aβ species with a longer carboxy terminus, which express more neurotoxicity and are generated from APP cleavage by the aberrant activity of

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γ-secretase, including presenilin 1 or 2 (PS1/2) harboring a FAD mutation. Because substrate APP and all APP-cleaving enzymes are membrane proteins, Aβ generation is linked to the intracellular transport of APP. Some causes of sporadic AD, which does not harbor any causative mutations on *APP* and *PSEN* genes, may be due to the altered intracellular trafficking of APP and APP-cleaving membrane-associated enzymes.

APP is a type I transmembrane protein, existing in several isoforms (APP_{770}, APP_{751}, and APP_{695}) that arise by alternative slicing of a single *APP* gene. In the biosynthetic process of APP in neurons, APP is subject to *N*-glycosylation in the endoplasmic reticulum and *O*-glycosylation in the Golgi [2]. The mature *N*- and *O*-glycosylated APP is further phosphorylated in the cytoplasm and transported to nerve terminals by kinesin-1 [3-7]. In the late secretary pathway, APP is subject to proteolytic cleavage; therefore, APP intracellular transport and metabolism are closely related.

Alterations in intracellular trafficking of APP are thought to alter APP processing in terms of quality or quantity or both, which is suggested from the independent genome-wide association study of patients with AD, indicating that disease-related single polymorphisms are found in genes for endocytosis and membrane vesicular trafficking [8-11]. Intracellular transport of APP is largely regulated in its short cytoplasmic region, wherein many cytoplasmic proteins interact for regulating intracellular trafficking of APP or membrane microdomain localization [12-17]. Therefore, the functions of these cytoplasmic regulators may be aberrant in sporadic AD.

In contrast to those for sporadic AD, mutations of APP in familial AD are detected in extracellular plus transmembrane domains covering the Aβ region, and no mutations are observed in the cytoplasmic region, which is evolutionally highly conserved in amino acid sequence. Thus, some of the APP pathogenic mutations are available for cleavage by secretases. For example, the Swedish mutation K670N/M671L facilitates β-site cleavage by BACE1 to generate $Aβ^{1-XX}$, and the London mutation V717I alters γ-site cleavage by γ-secretase to increase $Aβ^{1-42}$ but not $Aβ^{1-40}$ [18, 19]. However, how a protective mutation located in the Aβ region affects the cleavage of APP by α-, β- and γ-secretases remains unknown.

Intracellular trafficking and metabolism of APP

Intracellular APP trafficking and metabolism are summarized in figure 1, and the secretase cleavage sites on APP are indicated in figure 2. In general, APP is primarily cleaved by APP α-secretases ADAM10 and ADAM17. This α-site cleavage of APP occurs at the peptide bond between Lys687 and Leu688, cleaving the Aβ region of APP to generate CTFα/C83. This APP cleavage largely occurs in the plasma membrane. The resultant CTFα/C83 is further cleaved by a γ-secretase complex that includes PS1 or PS2 to generate amino-terminally truncated Aβ, $Aβ^{17-XX}$, named with the p3 fragment. This p3 fragment is metabolically labile and difficult to detect in cerebrospinal fluid, even when large amounts of the p3 fragment are generated from APP *in vivo*. Therefore, α-cleavage of APP does not generate neurotoxic Aβ forms such as $Aβ^{1-42}$; thus this APP metabolism is considered the non-amyloidogenic or amyloidolytic pathway. A small percent of APP can escape this pathway and become subject to endocytosis, entering an intracellular endosomal pathway in which β-site APP-cleaving enzyme-1 (BACE1) cleaves APP in the acidic environment, largely at the β-site to generate CTFβ/C99. This CTFβ/C99 is further cleaved by the γ-secretase complex, as is CTFα/C83, to generate neurotoxic $Aβ^{1-XX}$. This metabolism is considered the amyloidogenic pathway of APP. Enhanced entry of APP into the amyloidogenic pathway along with attenuated entry of APP into the non-amyloidogenic/ amyloidolytic pathway is a predominant cause of AD pathogenesis [17, 20-22].

BACE1 cleaves APP at several sites

The majority of the BACE1 cleavage of APP occurs between Met671 and Asp672 (β-site cleavage) to generate CTFβ/C99. However, BACE1 also cleaves APP at Tyr681 and Glu682 (β′-site cleavage) to generate an N-terminally truncated Aβ [23, 24], and at Leu705 and Met706 (β″-site cleavage) to generate a C-terminally truncated Aβ [25] (Figure 2). A pioneer study using primary cultured neurons reported that murine APP generates predominantly N-terminally truncated $Aβ^{11-xx}$ lacking ten amino acids in the amino terminus [26]. It was concluded that murine BACE1 preferentially cleaves APP at the β′-site, generating C89/CTFβ′, rather than at the β-site to generate

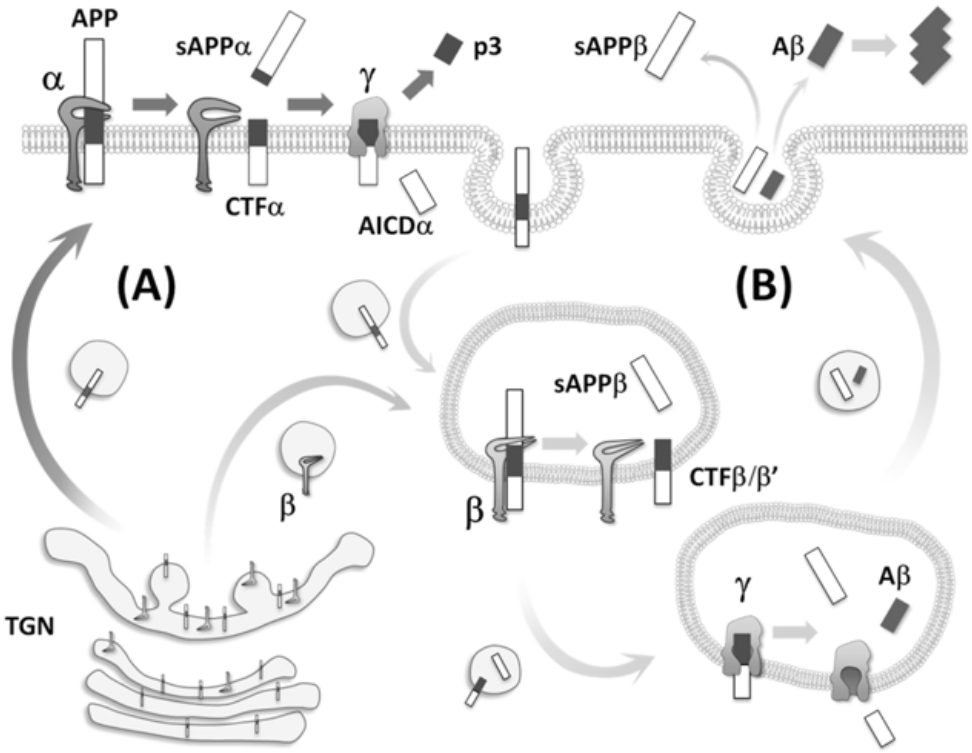

Figure 1. Schematic overview of APP intracellular transport and metabolism in neurons. The non-amyloidogenic or amyloidolytic pathway of APP. Mature APP with *N*- and *O*-glycosaccharides is subject to anterograde transport by kinesin-1 motor and reaches the plasma membrane. Large segments of APP are amyloidolytically cleaved by α-secretases (α) to generate sAPPα and CTFα/C83. CTFα/C83 is further cleaved by γ-secretase (γ) to secrete the p3 fragment along with the intracellular release of AICDα from the membrane. This is the non-amyloidogenic pathway (A). Some APP is subject to endocytosis and enters endosomal pathway in which acidic compartment BACE1 (β) is active to cleave APP at the β- or β'-site to generate CTFβ/C99 and CTFβ'/C89 along with sAPPβs. These CTFβs are further cleaved by γ-secretase to produce $Aβ^{1\text{-}xx}$ and $Aβ^{11\text{-}xx}$. The Aβs are secreted by exocytosis together with sAPPβs; therefore, this is the amyloidogenic pathway (B). Secreted Aβ forms neurotoxic oligomers to impair neuronal synapses.

Figure 2. Amino acid sequences in the Aβ region of human and mouse APP. Amino acids at three positions in the Aβ region of mouse APP (lower) differ from those of human APP (upper). Based on the amino acid numbering of the human APP_{770} isoform, Arg676, Tyr681, and His684 of human APP are Gly676, Phe681, and Arg684, respectively, in mouse APP. Icelandic Ala673Thr and Leuven Glu682Lys mutations of human APP are also indicated. Cleavage sites of APP by β-secretase (BACE1) as well as the peptide bonds between Met671 and Asp672 (β-site), between Tyr681 and Glu682 (β′-site), and between Leu705 and Met706 (β″-site) are shown with an α-secretase cleavage site (α-site) and typical γ-secretase cleavage sites (γ-sites).

C99/CTFβ. Although APP subject to cleavage by BACE1 in the intracellular compartment may differ in mice and humans, the completely conserved amino acid sequence of the APP cytoplasmic region among various animals, including a fish, conflicts with the idea of a species difference [27]. A recent report shows that mouse APP is cleaved at the β′-site rather than at the β-site in human or mouse neuroblastoma cells, whereas human APP is predominantly cleaved at the β-site in both human and mouse neuroblastoma cells [28]. This finding indicates that the difference in APP cleavage by BACE1 in humans and mouse is not dependent on the species specificity of BACE1, nor is it due to events in different intracellular compartments, at least when the amino acid substitutions are located within the amino-terminal half of the Aβ region (Figure 2).

There are three amino acid alterations within the amino-terminal half of the Aβ region in the mouse APP compared with the amino acid sequence of human APP. The differences are observed at position 676 (Arg in human is Gly in mouse; R676G), Y681F, and H684R. When His at position 684 of human APP is changed to the mouse Arg, the human APP harboring H684R shows mouse-type cleavage of APP preferentially at the β′-site, rather than the human-type cleavage predominant at the β-site (Figure 3). In other words, a single amino acid substitution in human APP can increase β′-site cleavage compared with β-site cleavage. The amino acid sequence-dependent preference of APP cleavage by BACE1, that is, a preferential choice of either β- or β′-site cleavage of APP by BACE1, is confirmed by an *in vitro* assay [28]. These data clearly indicate that the preference of

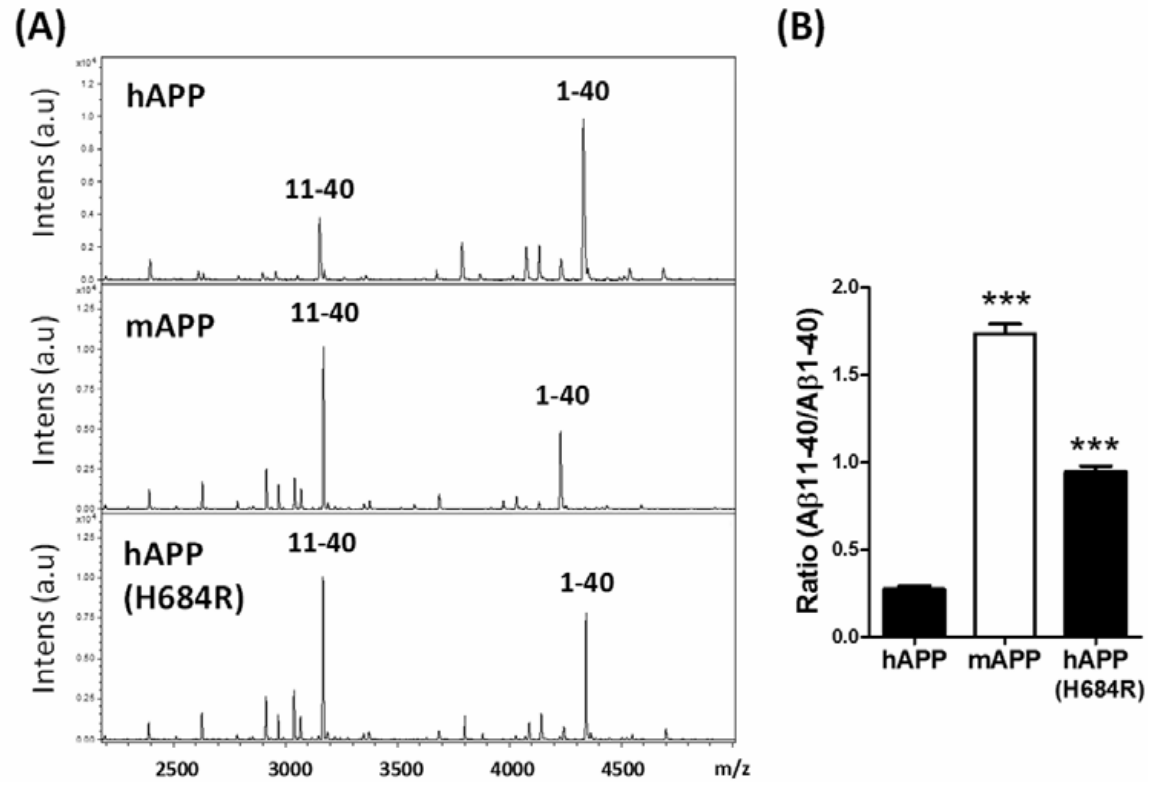

Figure 3. Amino acid position 684 plays an important role in determining whether BACE1 will cleave at the β- or β′-site of human APP. Human APP, mouse APP, and the human APP harboring the H684R (mouse type) amino acid substitution were expressed in mouse neuroblastoma N2a cells. Secreted Aβ forms were analyzed by immunoprecipitation–MS. (A) Representative MS spectra of Aβ in the medium. (B) The $A\beta^{11\text{-}40}/A\beta^{1\text{-}40}$ ratio was determined (n = 5; ± S.E.). The statistical significance of the differences between the human APP and the mouse APP and human APP H684R was determined using Dunnett's multiple comparisons test (***$p < 0.001$).

β- or β′-site cleavage of APP by BACE1 depends on the amino acid sequence of the Aβ region rather than the species specificity of BACE1 or cleavage in different intracellular compartments [28].

Pathogenic and protective mutations within the amino-terminal half of the Aβ region affect the selection of β- or β′-site cleavage of APP by BACE1

First, we focus on the Icelandic protective (A673T) and pathogenic (A673V) mutations, which are located close to the β-cleavage site, and the Leuven pathogenic mutation (E682K) within the β′-cleavage site, which cuts a peptide bond between Y681 and E682 [29-31] (Figure 2). The Leuven E682K mutation increases $Aβ^{1-XX}$ secretion and enhances production of CTFβ/C99 in cells [30]. Interestingly, APP harboring the Leuven E682K mutation shows markedly fewer β′-cleaved products ($Aβ^{11-XX}$) along with a decrease in CTFβ′/C89 (Figure 4), indicating that the Leuven E682K mutation attenuates the β′-cleavage of APP, as compared with the increased β-site cleavage of APP, to generate $Aβ^{1-XX}$. This is the reason that the Leuven E682K mutation functions pathogenically.

Next, we look at the APP with a mutation at position 673, which is located two amino acids from the β-cleavage site on the carboxy-terminal side (Figure 2). APP harboring A673V shows markedly increased cleavage at the β-site to generate CTFβ/C99, whereas the presence of A673T significantly decreases the production of CTFβ/C99 (Figure 5A). Furthermore, APP harboring A673V decreases the generation of $Aβ^{11-XX}$, whereas APP harboring A673T increases $Aβ^{11-XX}$ production. Taken together, the relative ratio of $Aβ^{11-XX}$ to $Aβ^{1-XX}$ ($Aβ^{11-XX}/Aβ^{1-XX}$) significantly decreases with the pathogenic A673V mutation, but markedly increases with the protective A673T mutation (Figure 5B) along with decreased secretion of $Aβ^{1-40}$ and $Aβ^{1-42}$ (Figure 5C). This result indicates that the molecular mechanism for the protective mutation at position 673 of APP is due to the enhancement of APP β′-site cleavage by BACE1, along with the attenuation of β-site cleavage by the same enzyme [28]. For the amino acid substitution at position 673 of APP, it is reasonable to think that the preference of APP β- or β′-site cleavage by BACE1 is determined by the amino acid sequence of the Aβ region rather than a species difference in BACE1 or in the different intracellular compartments where APP is cleaved.

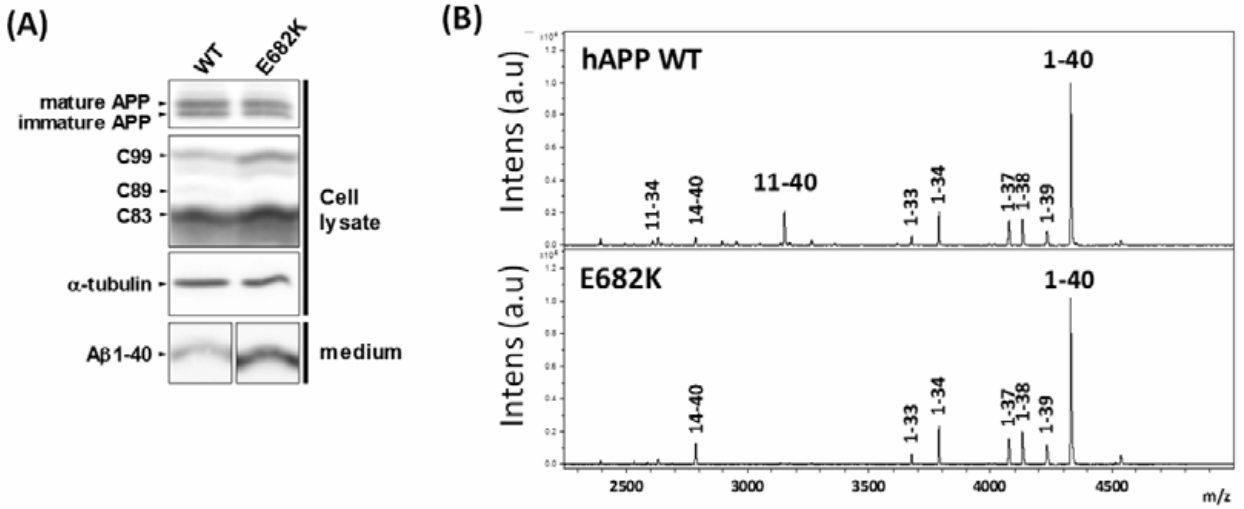

Figure 4. Attenuated β'-site cleavage by BACE1 of the human APP harboring the pathogenic Leuven E682K mutation. Human APP wild-type (WT) and APP E682K were expressed in N2a cells. (A) CTFβ/C99, CTFβ'/C89, and CTFα/C83 in cell lysates and $Aβ^{1-40}$ in media were analyzed by Western blotting followed by immunoprecipitation. (B) Aβ forms in the medium were also analyzed by immunoprecipitation–MS to detect $Aβ^{11-XX}$ forms.

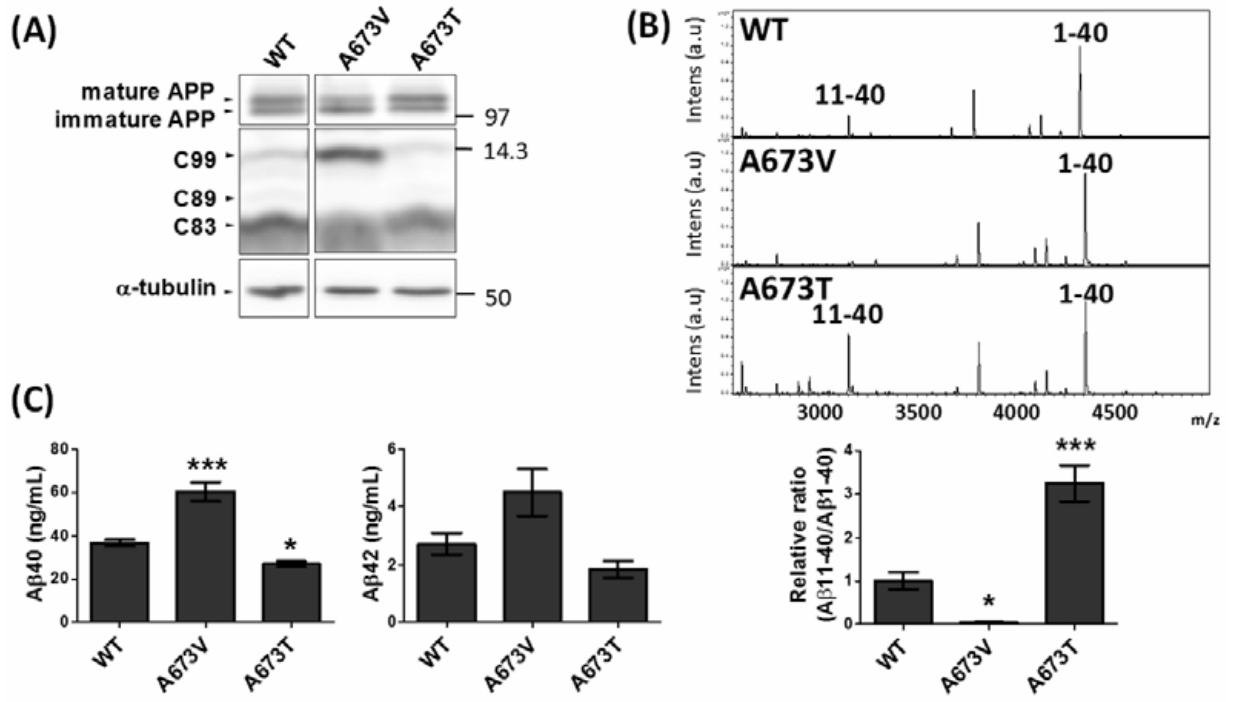

Figure 5. Enhanced β'-cleavage by BACE1 of the human APP harboring the protective Icelandic A673T mutation. Human wild-type (WT) APP as well as pathogenic (A673V) and protective (A673T) APP mutations were expressed in N2a cells. (A) CTFβ/C99, CTFβ'/C89, and CTFα/C83 in cell lysates were analyzed by Western blotting. (B) Aβ forms in the medium were analyzed by immunoprecipitation–MS to detect $Aβ^{11\text{-}XX}$ forms, and the $Aβ^{11\text{-}40}/Aβ^{1\text{-}40}$ ratios were determined (n = 5; ± S.E.). (C) $Aβ^{1\text{-}40}$ and $Aβ^{1\text{-}42}$ in the medium were also quantified by sELISA. The statistical significance of the differences between WT APP and APP A673V and APP A673T was determined using Dunnett's multiple comparisons test (*$p < 0.05$; ***$p < 0.001$).

The neurotoxicity of the amino-terminally truncated Aβ forms, including $Aβ^{11\text{-}40}$ and/or $Aβ^{11\text{-}42}$, is controversial [32, 33]. However, compared with $Aβ^{1\text{-}40}$ and $Aβ^{1\text{-}42}$, the truncated forms are metabolically labile *in vivo* and *in vitro* [28]. Murine primary cultured neurons generate $Aβ^{11\text{-}XX}$ predominantly from endogenous APP [26, 28]; however, the amount of $Aβ^{11\text{-}XX}$ in cerebrospinal fluid is lower than that of $Aβ^{1\text{-}XX}$. Even if the $Aβ^{11\text{-}XX}$ forms show weaker neuronal toxicity, the molecules are subject to faster degradation or clearance *in vivo*, diminishing their neuronal toxicity.

CONCLUDING REMARKS

The protective effect of the Icelandic APP A673T mutation against AD is largely due to enhanced cleavage of APP at the β′-site along with the attenuated cleavage of APP at the β-site. This β′-site cleavage of APP generates amino-terminally truncated $Aβ^{11\text{-}XX}$ forms, followed by γ-secretase cleavage of CTFβ′/C89. This conclusion may conflict with an expectation that the mutations alter the intracellular trafficking of APP. However, in the case of familial AD with mutations in the amino-terminal half of the Aβ region of APP, the choice for β- or β′-site cleavage of APP by BACE1 is largely dependent on the amino acid sequence of substrate APP. In sporadic AD without any mutations of the *APP* and *PSEN* genes, alterations in the intracellular transport of APP and APP-cleaving enzymes may play an important role in the pathogenesis, including enhanced Aβ generation and/or facilitated production of more neurotoxic Aβ forms such as $Aβ^{1\text{-}42}$. Furthermore, the $Aβ^{11\text{-}XX}$ forms generated by β′-cleavage are metabolically more labile than the $Aβ^{1\text{-}XX}$ forms in living neurons *in vivo* and may be subject to faster clearance prior to neurotoxic oligomerization, even if $Aβ^{11\text{-}XX}$ still possesses some neurotoxicity.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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