β- and γ-Secretases and Lipid Rafts

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Abstract: The cerebral accumulation of β-amyloid protein (Aβ) is thought to play a key role in the molecular pathology of Alzheimer's disease (AD). Recent evidence indicates that both β-secretase and γ-secretase, the membrane-associated proteases directly involved in the generation of Aβ from its precursor, amyloid precursor protein (APP), are localized to cholesterol-rich membrane microdomains termed lipid rafts. This underscores the significance of lipid rafts in the amyloidogenic processing of APP. In the present mini-review, I summarize recent research developments that shed light on the association of β-secretase and γ-secretase with lipid rafts, and discuss their implications for the pathology and therapeutics of AD.

Keywords: Alzheimer's disease, amyloid precursor protein, β-amyloid, BACE1, cholesterol, lipid raft, γ-secretase.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia in the elderly population. Recent evidence suggests that cerebral accumulation of β-amyloid protein (Aβ) plays a crucial role in the molecular pathology of AD [1]. Aβ is a hydrophobic peptide of 40–42 amino acids derived from the transmembrane amyloid precursor protein (APP). The processing mechanism that generates Aβ from APP, described below, is well characterized. The two proteases involved in this mechanism, β-secretase and γ-secretase, have attracted particular attention because they are considered potential therapeutic targets in AD [2,3].

Lipid rafts are distinct membrane domains characteri-zed by high concentrations of cholesterol and glycosphingolipids [4,5]. Lipid rafts are insoluble in non-ionic detergents and can be isolated as floating buoyant fractions by sucrose density gradient centrifugation; thus, cellular fractions enriched for lipid rafts are described in the literature by acronyms such as DRM (detergent-resistant membrane) and DIM (detergent-insoluble membrane). Lipid rafts play a central role in a number of cellular processes, including membrane sorting, trafficking, and signal transduction [4,5]. In addition, lipid rafts appear to be important in the pathogenesis of AD, reflecting the localization of both β- and γ-secretases to such rafts, and the involvement of rafts in the aggregation and accumulation of Aβ [6]. Therefore, lipid rafts are regarded as important in the context of the pathogenic mechanisms of AD. In this mini-review, I summarize the results from a number of studies describing associations of β- and γ-secretases with lipid rafts, and discuss their implications for the pathology and therapeutics of AD.

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SECRETASES AND Aβ

Amyloidogenic APP Processing

APP is first processed by β-secretase, generating a secreted derivative of APP (sAPP-β) and a β-C-terminal fragment (β-CTF). APP is alternatively processed by α-secretase within the Aβ region, generating sAPP-α and α-CTF. β-secretase has been identified as an aspartyl pro-tease, and is designated as β-site APP cleaving enzyme 1 (BACE1) [3,7,8]. α-secretase cleavage is most likely mediated by members of the ADAM (a disintegrin and metalloproteinase) family of proteases [9]. Subsequent cleavage of β-CTF within the membrane by γ-secretase generates Aβ (Aβ40 and Aβ42) and the APP intracellular domain (AICD). γ-secretase has been demonstrated to be a new type of aspartyl protease, forming a multi-protein complex in which presenilin 1 (PS1) or presenilin 2 (PS2) constitutes the catalytic subunit [10,11].

Characteristics of BACE1

BACE1 is a type 1 transmembrane protein composed of an N-terminal ectodomain, a transmembrane domain, and a short cytoplasmic domain. The ectodomain of BACE1 consists of a signal peptide, a prodomain, and a catalytic domain that contains four N-glycosylation sites. During maturation of BACE1, the prodomain is processed by furin, after which the protein is subjected to post-transla-tional modifications, such as glycosylation, phosphoryla-tion, and palmitoylation [7,8]. After maturation, BACE1 is initially targeted to the plasma membrane; subsequently BACE1 is internalized to endosomes. The dileucine motif in the cytoplasmic tail plays an important role in this internalization process. After internalization, BACE1 appa-rently cycles between the endosomal system, the *trans*-Golgi network (TGN), and the cell surface. BACE1 has an acidic optimum pH and is likely to be active in acidic compartments such as endosomes and the TGN. BACE1 is also partially cleaved by α-secretase-like proteases, leading to extracellular release of the ectodomain, although the physiological significance of this event is unclear [12,13]. Interestingly, biochemical characterization of BACE1

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revealed that BACE1 exists as a homodimer in cultured cells as well as in brain tissues [14,15]. Because Aβ pro-duction is abolished in BACE1 knockout mice, and such mice exhibit, at worst, only mild phenotypes, BACE1 is considered to be an excellent therapeutic target for anti-amyloid therapy [3,8].

Characteristics of γ-Secretase

γ-secretase is a high-molecular-weight complex comp-osed of PS (PS1 or PS2), nicastrin (NIC), APH-1, and PEN-2. PS1 and PS2 are membrane proteins containing nine transmembrane domains [10,11]. These proteins nor-mally undergo endoproteolysis between transmembrane domains 7 and 8, generating stable N-terminal and C-ter-minal fragments. APH-1 and PEN-2 are transmembrane proteins containing seven and two transmembrane dom-ains, respectively. NIC is a type 1 transmembrane protein that appears to function as a γ-secretase substrate receptor [16]. Numerous familial AD-associated mutations of the PS1 and PS2 genes have been reported and shown to affect amyloidogenic processing of APP, resulting in the genera-tion of higher amounts of the highly amyloidogenic Aβ42 relative to Aβ40. The fact that a number of type 1 trans-membrane proteins, including Notch, are also substrates of γ-secretase, complicates the development of clinically useful γ-secretase inhibitors [10,11].

β-SECRETASE AND LIPID RAFTS

Lipid Raft Localization of BACE1

Several studies have demonstrated that a considerable proportion of overexpressed BACE1 is present in lipid rafts [17-20]. Our analysis of endogenous BACE1 also indicated that mature BACE1 is mainly distributed in raft fractions, whereas immature BACE1 is localized to non-raft fractions [21]. Disrupting the integrity of lipid rafts by cholesterol depletion inhibits β-cleavage of APP, shifting BACE1 from raft to non-raft fractions [17]. Ehehalt and colleagues reported that increasing the association of APP with BACE1 through antibody cross-linking stimulated Aβ production in a cholesterol-dependent manner [22]. Using a mutant form of BACE1 in which the transmembrane and cytoplasmic domains were replaced with a glycosylphos-phatidylinositol (GPI) anchor attachment signal, Cordy and associates showed that the resultant mutant BACE1 was exclusively associated with lipid rafts and exhibited increased, cholesterol-dependent, β-cleavage activity [23]. These findings indicate that amyloidogenic processing of APP by BACE1 takes place more efficiently when BACE1 is localized in lipid rafts. BACE1 may also process other substrates, including neureglin-1 [24,25], P-selectin glyco-protein ligand-1 [26], lipoprotein receptor-related protein (LRP) [27], or β-subunits of voltage-gated sodium chan-nels [28] in lipid rafts.

Palmitoylation of BACE1 Regulates Lipid Raft Targeting

Post-translational modification by palmitoylation, which plays an essential role in raft localization of several membrane proteins [29], may act as a targeting signal for BACE1 raft localization. This possibility was recently addressed by Thinakaran's group. These authors observed that four cysteine residues (Cys474, Cys478, Cys482, and Cys485) at the junctions of transmembrane and cytoplas-mic domains are palmitoylated, and showed that raft localization of a palmitoylation-deficient mutant BACE1 containing Cys-to-Ala substitutions at these four sites was markedly reduced, as illustrated in Fig. (**1**) [30]. This find-ing has also been confirmed in our laboratory (unpublished observations). Therefore, palmitoylation at these four cysteine residues appears to mediate lipid raft localization of BACE1. Interestingly, however, BACE1-mediated pro-cessing of APP and Aβ production were reported to be unaffected by the absence of palmitoylation, suggesting that palmitoylation-deficient mutant BACE1 is capable of processing APP in non-raft domains. However, because the cells used in the cited study overexpressed APP, it remains to be determined whether a lack of BACE1 palmitoylation influences endogenous Aβ production.

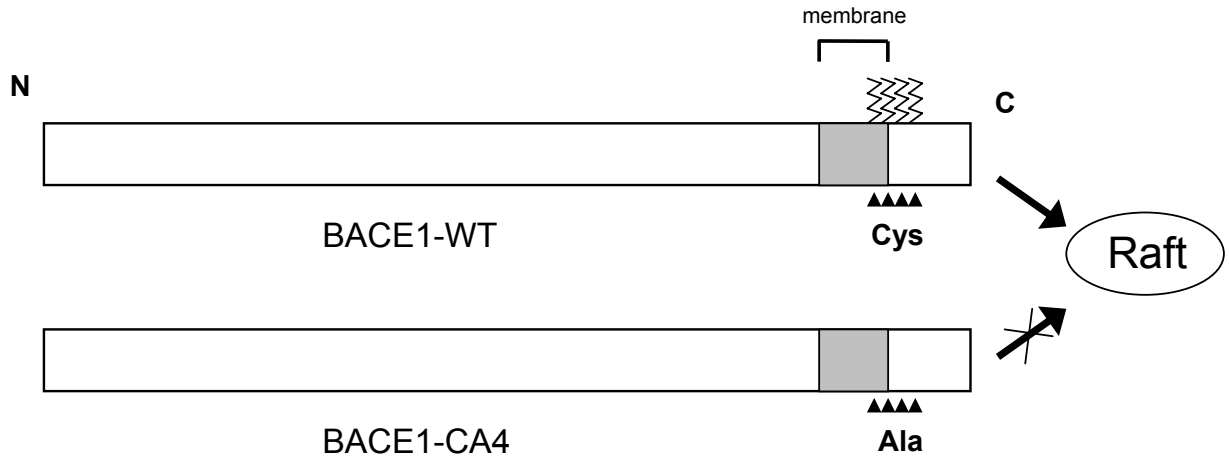

Fig. (1). Targeting of BACE1 to lipid rafts is regulated by palmitoylation.

Wild-type BACE1 (BACE1-WT) is palmitoylated at cysteine residues Cys474, Cys478, Cys482, and Cys485. Mutant BACE1 (BACE1-CA4), in which these cysteines are substituted with alanines, is not palmitoylated. Raft localization of BACE1-CA4 is markedly reduced relative to BACE1-WT, suggesting that palmitoylation mediates targeting of BACE1 to rafts [30].

Regulation of BACE1 in Lipid Rafts

How the β-cleavage of APP by BACE1 is regulated in lipid rafts is not yet known. It has been shown that BACE1 activity is negatively regulated by reticulon (RTN) family proteins, such as RTN3 and RTN4-B/C (or Nogo-B/C), which physically interact with BACE1 [31,32]. However, we observed that reticulons are predominantly localized to non-raft fractions (unpublished observations), and are thus unlikely to participate significantly in the regulation of BACE1 in lipid rafts. Parkin and co-workers reported that the normal cellular form of the prion protein (PrP^C) interacts with BACE1 and regulates the β-cleavage of APP [33]. These authors found that the polybasic N-terminal region of PrP^C and localization of PrP^C to lipid rafts are required for the inhibitory effect of PrP^C on β-secretase-mediated cleavage of APP. The cited authors suggested that, within a subset of lipid rafts, the N-terminus of PrP^C may interact via glycosaminoglycans with one or more of the heparin-binding sites on BACE1, thereby restricting access of BACE1 to APP.

Nukina and co-workers have proposed an interesting mechanism to account for APP-BACE1 interaction in rafts [34]. Their data suggest that APP and BACE1 are segregated into distinct microdomains. According to their mechanism, APP is associated with syntaxin 1-containing microdomains, but upon neuronal activation, the APP microdomain association is switched from syntaxin 1 to BACE1, thus promoting APP-BACE1 interaction. Although this "microdomain switching" mechanism is attractive, the copatching technique used in these experiments labels both APP and BACE1 at the cell surface, and the study featured extensive use of a BACE1 mutant lacking the C-terminal endocytosis signal. Accordingly, how APP and native BACE1 are associated in lipid rafts on endosome membranes remains to be clarified.

A recent study by Kang and co-workers showed that the cytoplasmic domain of LRP promotes the localization of APP and BACE1 to lipid rafts and their physical association, increasing APP β-CTF levels and Aβ secretion [20]. Their data also indicate that endogenous LRP is required for the normal trafficking of APP to lipid rafts and Aβ generation. Moreover, the same group reported that Ran-binding protein 9, which interacts with LRP, facili-tates APP association with lipid rafts and Aβ generation [35]. It is thus likely that APP-BACE1 interaction in lipid rafts is modulated by LRP and other LRP-associated molecules.

γ-SECRETASE AND LIPID RAFTS

Lipid raft Localization of γ-Secretase

γ-secretase has been shown to be tightly associated with lipid rafts. For example, fractionation analyses of cultured cells and brain tissues have shown that all components of γ-secretase are primarily localized to the lipid raft fraction [21,36-39]. The four components of γ-secretase complex are stable in rafts, even at high concentrations of detergents such as CHAPSO. Moreover, the lipid raft fraction con-tains high levels of γ-secretase activity, based on assay of AICD production [36,39]. Thus, γ-secretase is enriched in lipid rafts, although its substrate levels are relatively low. According to a report by Thinakaran's group, γ-secretase is mainly localized to lipid raft microdomains of post-Golgi and endosomes [37].

Thinakaran's group has also presented evidence that NIC is palmitoylated at Cys689, and APH-1 is palmitoy-lated at Cys182 and Cys245 [40]. Using palmitoylation-deficient mutants, the cited authors showed that palmitoy-lation of NIC and APH-1 contributes to raft association of NIC and APH-1. This reaction is also important for NIC and APH-1 stability, but does not directly modulate processing of substrates by γ-secretase.

Cholesterol, Statins, Protein Isoprenylation, and γ-Secretase

Depletion of cholesterol by methyl-β-cyclodextrin, a cholesterol-sequestering reagent, disrupts lipid raft inte-grity. In the presence of methyl-β-cyclodextrin, the four components of γ-secretase become dissociated from lipid rafts and are redistributed to non-raft domains, indicating that the association of γ-secretase with lipid rafts is cholesterol-dependent [36-38]. Statins, which are 3-hydroxy-3-methylglutaryl-CoA-reductase inhibitors with choles-terol-lowering properties, were also reported to decrease the association of the γ-secretase complex with lipid rafts. This effect was partially abrogated by the addition of geranylgeraniol, suggesting that both cholesterol and protein isoprenylation influence the association of γ-secretase with lipid rafts [38]. Consistent with this finding, geranylgeraniol treatment was shown to increase active γ-secretase in lipid rafts (along with its substrate, APP CTFs) as well as γ-secretase–mediated Aβ42 production [41]. However, the mechanism by which protein isoprenylation mediates incorporation of γ-secretase into lipid rafts remains unknown.

IMPLICATIONS FOR THE PATHOLOGY AND THERAPEUTICS OF AD

Cholesterol, Lipid Rafts and Secretases

Accumulating evidence suggests that cholesterol may play a role in AD [42]. Results obtained both in vitro and from animal studies indicate that cholesterol levels modulate Aβ production and accumulation. For example, APP/PS1 double-transgenic mice fed a high-lipid diet have increased cholesterol levels in the central nervous system and exhibit an accelerated amyloid pathology [43]. In contrast, the same mice treated with a cholesterol-lowering drug exhibit a reduced Aβ pathology [44]. An in vitro study also showed that cholesterol reduction independently inhibits both β-secretase and γ-secretase [45]. A direct correlation between cholesterol levels and BACE1 expression levels in neurons has also been reported [46].

It is not clear how cholesterol levels modulate β- and γ-secretase activities. Recent studies employing a recons-tituted system composed of purified proteins and defined lipids have provided evidence that membrane lipid composition is important in modulating secretase activities [47,48]. Particularly, cholesterol has been shown to have stimulating effects on both BACE1 and γ-secretase activities [47,48], and BACE1 activity is dramatically reduced

by cholesterol depletion [47]. Therefore, it is possible that β- and γ-secretase activities can be modified by altering cholesterol levels in the brain. More research is needed to elucidate the relationship between the lipid composition of membrane lipid rafts and β- and γ-secretase activities *in vivo*.

Oxidative Stress and Secretases in Lipid Rafts

Oxidative stress is considered to be an important factor in the pathogenesis of AD [49,50]. In particular, there is a positive feedback relationship between oxidative stress and Aβ: Aβ promotes oxidative stress, which in turn, enhances production of Aβ. Several recent reports indicate that oxidative stress affects BACE1 and γ-secretase [51-54]. However, the relationship between oxidative stress and the activities of these proteases in lipid rafts remains to be clarified. Our laboratory recently addressed this issue by exposing human neuroblastoma cells to ethacrynic acid (EA), which induces oxidative stress via glutathione depletion [21]. We showed that EA treatment caused a significant increase in PS1 mRNA expression and increased PS1 protein levels in both cell lysates and the lipid raft fraction without altering BACE1 or other γ-secretase components. EA treatment also promoted Aβ secretion from cells expressing Swedish mutant APP. A vicious cycle may thus exist between Aβ and oxidative stress, wherein Aβ triggers oxidative stress, which up-regulates PS1 protein in lipid rafts, and consequently promotes Aβ production. The use of anti-oxidants to break this cycle may prove to be beneficial as a therapeutic intervention for AD.

Development of Secretase Inhibitors

Simons and co-workers prepared a sterol-linked β-secretase inhibitor and showed that the material exhibited more potent inhibitory activity than did the free inhibitor [55]. The cited authors also showed that the sterol-linked inhibitor was internalized into endosomes containing APP/BACE1 and was readily partitioned into raft domains. Furthermore, stereotaxic injection of the sterol-linked inhibitor into the hippocampus of APP/PS1 transgenic mice effectively inhibited Aβ production. Thus, membrane-anchoring of the β-secretase inhibitor can increase its potency, most likely by enhancing the interaction between the inhibitor and BACE1. Such a membrane-targeting strategy might be useful in the design of clinically applicable BACE1 inhibitors.

CONCLUDING REMARKS

The predominant localization of mature BACE1 and γ-secretase in lipid rafts is indicative of the significance of such rafts as the site of Aβ production. However, there are many unresolved issues surrounding the issue of lipid-raft association of secretases. *In vitro* studies have pointed to important roles for the lipid microenvironment in the modulation of β- and γ-secretase activities. However, the precise relationship between the lipid microenvironment and secretases *in vivo* is still unclear. Moreover, how the activity of BACE1 is regulated in lipid rafts and how the interaction of BACE1 and APP is controlled in rafts remain poorly understood. Elucidating the mechanisms involved in controlling the interaction of APP CTFs and γ-secretase in lipid rafts is also a subject for future research. Ultimately, it is hoped that resolving these and other issues will provide useful clues for the development of new therapeutic strategies to manage AD.

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