Dual Role of α-Secretase Cleavage in the Regulation of γ-Secretase Activity for Amyloid Production

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Yuan Tian†‡§, Christina J. Crump†‡, and Yue-Ming Li†‡¶

From the †Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10065 and the ‡Program of Physiology, Biophysics, and Systems Biology and ¶Department of Pharmacology, Joan Well Graduated School of Medical Science of Cornell University, New York, New York 10021

Processing of the amyloid precursor protein (APP) by β- and γ-secretases generates pathogenic β-amyloid (Aβ) peptides associated with Alzheimer disease (AD), whereas cleavage of APP by α-secretases precludes Aβ formation. Little is known about the role of α-secretase cleavage in γ-secretase regulation. Here, we show that α-secretase-cleaved APP C-terminal product (αCTF) functions as an inhibitor of γ-secretase. We demonstrate that the substrate inhibitory domain (ASID) within αCTF, which is bisected by the α-secretase cleavage site, contributes to this negative regulation because deleting or masking this domain turns αCTF into a better substrate for γ-secretase. Moreover, α-secretase cleavage can potentiate the inhibitory effect of ASID. Inhibition of γ-secretase activity by αCTF is observed in both in vitro and cellular systems. This work reveals an unforeseen role for α-secretase in generating an endogenous γ-secretase inhibitor that down-regulates the production of Aβ. Deregulation of this feedback mechanism may contribute to the pathogenesis of AD.

The amyloid precursor protein (APP) ² is sequentially cleaved by β- and γ-secretases to generate Aβ peptides, which are widely considered to play a causative role in the pathogenesis of Alzheimer disease (AD) (1). This pathway also generates soluble APPβ (sAPPβ) and APP intracellular domain. Alternatively, APP can be processed by α-secretase that cuts within Aβ peptides, which precludes the formation of toxic peptides. Similarly, the α/γ pathway leads to formation of soluble APPα (sAPPα), the P3 peptide, and the APP intracellular domain. It appears that γ-secretase cleavage of substrates first requires another protease that removes the majority of the extracellular domain of the substrate. The sequential cleavage of APP is characteristic of regulated intramembrane proteolysis (RIP) (2). RIP generally requires two proteolytic steps, whereby the second intramembrane cleavage is dependent on the first cleavage, such as in Notch and sterol regulatory element binding proteins signaling. RIP has been found to govern sterol regulation, cell fate determination, unfolded protein responses, growth factor activation, mitochondria membrane remodeling, and apoptosis (2–5).

A distinctive feature of APP processing compared with other RIPs is the existence of two proteases (β- and α-secretase) that are both capable of executing the first cleavage. Recent studies have shown that β-secretase cleavage removes the large extracellular domain of APP, freeing the N terminus of βCTF, which is recognized by nicastrin (6). Recruiting βCTF to the docking site makes the substrate accessible to the active site of γ-secretase for hydrolysis. However, the role of nicastrin for substrate binding has been controversial (7–9). Nevertheless, β-secretase cleavage converts a latent substrate of γ-secretase into an active one. Whether α-secretase plays a role similar to that of β-secretase in the regulation of APP processing by γ-secretase is unknown. It has been suggested that α-secretase competes with β-secretase for the APP substrate and thereby reduces the production of Aβ (10–13). Therefore, elevation of α-secretase activity has been suggested as a therapeutic strategy to reduce the production of Aβ (14). Also, it has been reported that AD patients have reduced levels of α-secretase activity (as reflected by sAPPα levels in cerebrospinal fluid) relative to that of healthy controls, but there was no difference in the level of sAPPβ (15, 16), which results from β-secretase cleavage. These observations of reduced sAPPα levels together with normal sAPPβ levels in certain populations of AD patients indicates that α-secretase can modulate the rate of Aβ production without competing with β-secretase. In addition, transgenic mouse studies showed that α-secretase competes for APP substrate with β-secretase when ADAM10, an α-secretase candidate, was highly overexpressed (13). However, when a moderate level of ADAM10 was expressed in mice, Aβ was reduced, and no statistical difference of sAPPβ between transgenic and control animals was detected (13), suggesting that there is no significant competition for the APP substrate between α- and β-secretase. These studies suggest that α-secretase can modulate Aβ production by an undefined mechanism in addition to depletion of the APP substrate. Recently, we identified a substrate inhibitory domain (ASID) within βCTF that negatively modulates γ-secretase activity for Aβ production (17). Furthermore, the APP Flemish mutation within ASID sequence reduces its inhibitory effect on γ-secretase, resulting in...
increased Aβ production (17). After α-secretase cleavage, ASID becomes exposed at the N terminus of α-CTF. How ASID within αCTF modulates γ-secretase is unknown and whether this domain is involved in γ-secretase activity for Aβ production remains to be explored.

In this study, we have demonstrated that α-secretase cleavage of APP generates αCTF that functions as a γ-secretase inhibitor for Aβ production. Our studies suggest that ASID within αCTF is responsible for this negative regulation. Therefore, α-secretase plays a dual role in modulation of γ-secretase for Aβ production.

**EXPERIMENTAL PROCEDURES**

**Expression of Biotinylated Recombinant Proteins**—AviTag, a specific peptide sequence that can be biotinylated with biotin ligase, was cloned into a pIAD16 vector (18) to generate a pIAD16Avi plasmid. APP fragments with FLAG tag were inserted into the pIAD16Avi vector. For expression of biotinylated protein, pIAD16Avi-APP and pACYC164, which encodes biotin ligase, were co-transformed in BL21 (DE3) cells. When bacteria growth reached 0.4–0.8 at A600, isopropyl 1-thio-β-D-galactopyranoside (100 μM) was added to induce target protein expression in the presence of 50 μM biotin. Cells were pelleted and lysed by French press. The soluble fraction was subjected to standard amylose affinity chromatography through a maltose-binding protein (MBP) tag. The purified protein was treated with thrombin at 16 °C overnight to cleave between the MBP tag and AviTag. The biotinylation of target proteins was verified by LC-MS/MS.

**In Vitro γ-Secretase Assay**—The recombinant proteins were incubated with γ-secretase (40 μg/ml) in the presence or absence of 1 μM γ-secretase inhibitor L-685,458. The assays were performed as previously described (17, 19, 20) using electrochemiluminescence (ECL) technology. The amount of product was determined using synthetic peptide assays.

**Cell-based Aβ Production Assay**—HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum and penicillin.
peptides were detected by ECL assay using biotinylated 6E10 or biotinylated 4G8 and ruthenylated G2-10 antibodies (20). Conditioned media and cell lysates were analyzed by Western blotting using sAPPβ, 6E10, W0-2, and CT15 antibodies (see corresponding figures for antibody recognition site of APP).

**Immunoprecipitation and βCTF ECL Assay**—The mock (empty vector) or tumor necrosis factor-α-converting enzyme (TACE)-transfected HEK293-APP cell lysate was collected and lysed with 200 μl of radioimmunoprecipitation assay buffer with a mixture of protease inhibitors. After rotating at 4°C for 1 h, cell lysates were centrifuged at 13,000 rpm for 5 min, and the supernatants were subjected to immunoprecipitation with W0-2 or 4G8 antibody and blotted with varying antibodies.

The mock- or TACE-transfected HEK293-APP cell lysate were also incubated with biotinylated 6E10, APPc, and ruthenylated anti-rabbit secondary antibodies for 3 h; magnet streptavidin beads were then added and incubated for 30 min. The assay mixtures were analyzed by ECL technology.

**RESULTS**

**Expression of α-Secretase-like Activity Causes an Accumulation of βCTF and a Reduction of Aβ in Cells**—To explore the undefined role of α-secretase in regulation of γ-secretase activity, we examined the effect of TACE on the processing of APP. Both ADAM10 and TACE have been found to exhibit α-secretase activity (21, 22). Therefore, the expression of TACE or ADAM10 is able to promote αCTF production. We expressed TACE fused with a HA tag in HEK293-APP cells and examined its effect on APP processing. We first confirmed TACE protein expression by Western blot analysis using an anti-HA antibody (Fig. 1A, top panel). The expression of TACE had only a moderate effect (15% reduction) on the level of APP (Fig. 1A, middle panel). Equal protein loading was confirmed by anti-β-tubulin Western blotting (Fig. 1A, bottom panel).

Although TACE can cleave multiple substrates, we focused on its action in APP processing. We analyzed the secreted APP species that include sAPPα, sAPPβ, and Aβ in conditioned media. sAPPα and sAPPβ, which refer to the liberated N-terminal fragments of APP resulting from the cleavages of α-secretase and β-secretase, were specifically recognized by the 6E10 and anti-sAPPβ antibodies, respectively. We found elevated sAPPα levels, indicating that the transiently expressed TACE increases α-secretase-like processing of APP. Furthermore, reduced sAPPβ suggested that there was less substrate available for β-secretase cleavage (Fig. 1B), which is a consequence of TACE-mediated depletion of the APP substrate. This finding is consistent with previous reports of competition between α-secretase and β-secretase for the APP substrate (10, 11, 13).

We next determined the level of secreted Aβ40 with 6E10 and G2-10 antibodies. The amount of Aβ40 was significantly reduced by 82% following TACE overexpression (Fig. 1C), which is consistent with previous results seen in ADAM10 mouse studies (13). However, when the total amount of γ-secretase-cleaved products including Aβ and P3, collectively known as X40, were examined, we found that up-regulation of TACE activity also resulted in a 53% reduction of X40 (Fig. 1C). Clearly, higher α-secretase activity leads to a reduction of total γ-secretase-cleaved products (P3 and Aβ40), suggesting that an increased production of αCTF suppressed overall γ-secretase activity for APP processing. In addition, we have determined the amount P3 in media using synthetic Aβ as a standard. We found that the expression of TACE led to a reduction of P3 by ~50% relative to vector control, supporting that γ-secretase activity for production of both Aβ and P3 is reduced.

To examine further this notion that αCTF functions as an endogenous inhibitor of γ-secretase in cells, we determined the effect of TACE expression on cellular αCTF and βCTF frag-

**Feedback Modulation of γ-Secretase**


**Feedback Modulation of γ-Secretase**

| substrate | sequence | γ-secretase activity % |
|-----------|----------|------------------------|
| Sb1       | KGAILGLMVGGYATVITLLMKKK | 100 ± 4.2 |
| Sb2       | LVFFAEQSGNKGAIGLWCGVYATVITLLMKKK | < 5 |
| Sb3       | LVFFAEQSGNKGAIGLWCGVYATVITLLMKKK | < 5 |
| Sb4       | VYSGNKGAIGLWCGVYATVITLLMKKK | 125 ± 6.2 |
| Sb5       | YVYHGCKGSKGAIGLWCGVYATVITLLMKKA | 85 ± 2.9 |
| Sb6       | KGAGNQGSKGAIGLWCGVYATVITLLMKK | 108 ± 5.2 |

![Graph](image)

**FIGURE 3. Deletion, mutation, or antibody-mediated masking of the inhibitory domain of αCTF restores its reactivity with γ-secretase.** A, γ-secretase activity of the αCTF-derived substrates. Gray rectangles represent the AviTag with the sequence GLNDIFEAQKIEWHE. γ-Secretase activity is monitored by in vitro γ-secretase activity assay as described in Fig. 2A using only the ruthenylated G2-10 antibody. β, inhibitory potencies of Aβ1–21 and Aβ1–21. C, effect of the Sb3 protein complexed with 4G8 antibody on γ-secretase activity in vitro. Monoclonal 4G8 antibody (100 ng/μl) was preincubated with Sb3 protein (1 μM) for 30 min. The biotinylated Sb3 with and without treatment of 4G8 was incubated with HeLa membrane in the presence of 0.25% CHAPS. The X401 product was detected using streptavidin beads and G2-10 antibody (mean ± S.E. (error bars), n > 3).

The level of both αCTF and βCTF was higher in the TACE-expressing cells than in control cells, suggesting a reduction of γ-secretase activity for βCTF processing (Fig. 1G).

Although the level of both αCTF and βCTF was higher in the TACE-expressing cells than in vector-control cells, the total γ-secretase-cleaved products, including both P3 and Aβ, were diminished, indicating that expression of α-secretase like activity could have dual effects on Aβ production. First, competing with β-secretase for APP substrate results in a reduction of Aβ. Second, αCTF functions as an endogenous inhibitor that reduces γ-secretase activity for Aβ production. This novel role of α-secretase is supported by the finding that increased α-secretase like activity leads to a reduction of γ-secretase activity for Aβ production and an accumulation of βCTF in cells despite a reduction in sAPPβ. However, whether functional inhibition of total γ-secretase activity is attributed to the ASID (17) within αCTF needs to be further investigated. Nevertheless, these studies suggest that αCTF is a poor substrate and/or an inhibitor that negatively modulates γ-secretase activity.

αCTF Is a Poor Substrate of γ-Secretase in Vitro—To compare the reactivity αCTF and βCTF with γ-secretase directly, we overproduced recombinant proteins as MBP fusion proteins with a thrombin cleavage site (Fig. 2A). Purified fusion proteins were treated with thrombin and analyzed by SDS-PAGE (Fig. 2B). The molecular mass of αCTF-FLAG and βCTF-FLAG was confirmed by LC-MS (Fig. 2C). The measured molecular mass of αCTF-FLAG and βCTF-FLAG was 10,621 and 12,557 kDa, respectively, and matched the calculated masses 10,617 and 12,545. Each substrate was incubated with HeLa membrane at three concentrations in the presence and absence of 1 mM L-685,458, a potent γ-secretase inhibitor (23). The signal difference between L-685,458-treated and untreated samples is attributed to γ-secretase activity. The Aβ40 site cleavage was detected by a pair of antibodies: biotinylated 4G8 and ruthenylated G2-10. The two substrates exhibit striking differences in their ability to be processed by γ-secretase. The rate of γ-secretase hydrolysis of βCTF-FLAG is 12-fold greater than for hydrolysis of αCTF-FLAG (Fig. 2D). This result indicates that αCTF is a poor substrate, which supports our assertion that αCTF can function as an endogenous γ-secretase inhibitor in cells.

**ASID within αCTF Regulates γ-Secretase Activity in Vitro**—Recently, we demonstrated that ASID within βCTF negatively regulates the activity of γ-secretase (17). This prompted us to determine whether this domain, which is located at the N terminus of αCTF, accounts for the poor reactivity of αCTF for γ-secretase. Therefore, we attempted to delete or mutate this domain to assess its role. However, alteration of this inhibitory
domain destroys the 4G8 antibody-binding epitope upon which our assay depends; hence, we introduced an AviTag into the N terminus of APP CTFs. AviTag, a specific 15-residue peptide, is recognized by biotin ligase that specifically catalyzes an attachment of biotin to the lysine residue within the AviTag. Biotinylation of the recombinant substrate allows us to monitor \( \gamma \)-secretase activity directly using streptavidin beads and the G2-10 antibody. To facilitate protein isolation, we inserted an MBP/thrombin site ahead of the AviTag on these target proteins. First, we tested this strategy by directly fusing the transmembrane domain of APP behind the MBP/thrombin-AviTag. A target protein was co-expressed with biotin ligase in the presence of biotin. The biotinylated APP transmembrane domain, which is hereafter referred to as Sb1, was confirmed by LC-MS analysis. The Sb1 protein was incubated with HeLa membrane, and the A\( \beta \)40 site cleavage was detected using the G2-10 antibody. \( \gamma \)-Secretase effectively cleaved the Sb1 substrate (Fig. 3A), and therefore we constructed two additional substrates, Sb2 and Sb3, which both contain the A\( \beta \)17–23 sequence, with Sb3 also including the C-terminal tail of APP (Fig. 3A). Very low 40-site specific \( \gamma \)-secretase cleavage product was detected using both the Sb2 and Sb3 substrates. However, deleting the ASID (A\( \beta \)17–23) as we previously reported generates a better substrate (Sb4) with a rate similar to that of the Sb1 substrate (Fig. 3A). Again, the critical question to address was whether the high reactivity of the Sb4 substrate was caused by shortening of the N-terminal portion of this \( \gamma \)-secretase substrate or by a sequence-specific effect as we observed in \( \beta \)CTF. Therefore, we designed two more constructs, Sb5 and Sb6. The A\( \beta \)17–23 sequence of Sb3 was replaced by A\( \beta \)10–16 (YEVHHQK) in Sb5 or a random sequence (VAGAGGN) in Sb6. Both Sb5 and Sb6 are remarkably active (Fig. 3A). Their rates of \( \gamma \)-secretase proteolysis were 0.85- and 1.1-fold of Sb1 for Sb5 and Sb6, respectively. This observation strongly indicates that it is the ASID in \( \alpha \)CTF that directly regulates \( \gamma \)-secretase activity, rather than an effect being mediated by shortening of the N-terminal portion of the substrate. Furthermore, it appears that removal of A\( \beta \)1–16 enhances the effect of ASID, indicating that the A\( \beta \)1–16 sequence may play a specific role in modulating the inhibitory effect of ASID because \( \beta \)CTF is a substrate of \( \gamma \)-secretase. To examine the relationship of A\( \beta \)1–16 and ASID, we synthesized A\( \beta \)1–16, A\( \beta \)1–7, A\( \beta \)1–12, A\( \beta \)8–16, and A\( \beta \)1–21 peptides and tested their effect on \( \gamma \)-secretase activity for processing of the Sb3 substrate. Combinations of three concentrations (0.1, 1, and 10 \( \mu \)M) of A\( \beta \)1–16 and three concentrations (0.5, 1, and 2 \( \mu \)M) of Sb3 were used to test two peptide motifs for \( \gamma \)-secretase activity in trans (acting from a different molecule). There is no difference among all of these combinations, i.e. little 40-site-specific \( \gamma \)-secretase product was detected. However, when we compared the inhibitory potency of A\( \beta \)1–21 and A\( \beta \)17–21, we obtained IC\(_{50}\) values for A\( \beta \)1–21 and A\( \beta \)17–21 of 7.8 \( \mu \)M and 0.3 \( \mu \)M, respectively (Fig. 3B). These studies suggest that the A\( \beta \)1–16 sequence only works in cis (acting from the same molecule) by coordinating with A\( \beta \)17–21 for regulation of \( \gamma \)-secretase activity. Furthermore, \( \alpha \)-secretase cleavage significantly enhances the inhibitory effect of ASID, indicating that \( \alpha \)-secretase-like cleavage can serve as a negative feedback for \( \gamma \)-secretase modulation.

![Feedback Modulation of \( \gamma \)-Secretase](image)

**FIGURE 4.** \( \alpha \)CTF negatively modulates \( \gamma \)-secretase activity in vitro. A, effect of recombinant \( \alpha \)CTF on \( \gamma \)-secretase activity. \( \alpha \)CTF (0.25, 0.5, and 1 \( \mu \)M) was added to the in vitro \( \gamma \)-secretase reaction with 1 \( \mu \)M \( \beta \)CTF substrate in the presence of 0.25% CHAPSO. Detection of X40 and A\( \beta \)40 was performed as explained in Fig. 1C (mean ± S.E. [error bars], n > 3). Dashed arrows represent changes of \( \gamma \)-secretase-cleaved products. As shown on the right, \( \alpha \)CTF inhibits \( \gamma \)-secretase activity for the processing of \( \beta \)CTF. B, effect of recombinant Sb4 on in vitro \( \gamma \)-secretase activity. Sb4 protein, which lacks the inhibitory domain, was added at concentrations of 0.25, 0.5, and 1 \( \mu \)M to the in vitro \( \gamma \)-secretase reaction mixture in the presence of 1 \( \mu \)M \( \beta \)CTF as substrate. The 40-site product was detected by 6E10/G2-10 antibodies (mean ± S.E., n > 3). As indicated by the scheme on the right, Sb4 does not suppress the processing of \( \beta \)CTF by \( \gamma \)-secretase and is even a better substrate of \( \gamma \)-secretase. C, effect of \( \alpha \)CTF on \( \gamma \)-secretase activity for Notch1 cleavage. The Notch1 cleavage was detected with a Notch1 cleavage-specific antibody 5M320 (mean ± S.E., n > 3).

We next examined whether masking ASID reduces its negative regulation of \( \alpha \)CTF processing and therefore increases substrate reactivity in vitro. To test this hypothesis, we preincubated the Sb3 substrate with the monoclonal antibody 4G8, which binds directly to the LVFFAE epitope and should therefore mask the inhibitory domain. The assay background was defined in the presence of L-685,458. After subtracting the L-685,458-defined background, there was a 26-fold increase in \( \gamma \)-secretase activity with the 4G8 antibody-bound substrate (Fig. 3C). Therefore, our data show that either deleting or masking the inhibitory domain of \( \alpha \)CTF alters its regulatory role and results in an increase in \( \gamma \)-secretase-cleaved products.
**Feedback Modulation of γ-Secretase**

![Diagram](image)

**FIGURE 5. Proposed model of the inhibitory domain in the regulation of γ-secretase for APP processing.** The figure shows the mechanism of action of the inhibitory domain when it is engaged with βCTF or αCTF. The interaction of αCTF with γ-secretase forms a low or unproductive complex that allows the inhibitory domain to modulate γ-secretase activity both in cis and in trans and thus leads to a reduction of γ-secretase activity for production of Aβ and P3. Concurrently, it results in an accumulation of βCTF. βCTF binds to γ-secretase to form a productive complex for production of Aβ. However, interaction of the ASID with the inhibitory domain binding site (E) of γ-secretase limits the catalytic ability of active site (EAS) for Aβ production in cis.

αCTF Inhibits γ-Secretase Activity for the Processing of βCTF in Vitro—To examine further the notion that ASID within αCTF is responsible for its poor reactivity as a substrate and αCTF functions as an endogenous γ-secretase inhibitor for Aβ production, we determined in vitro γ-secretase activity for the production of X40 (P3 + Aβ40) and Aβ40 in the presence of both the βCTF and αCTF substrates (Fig. 4A). At 0.25, 0.5, and 1.0 μM, αCTF significantly suppresses γ-secretase activity for Aβ production by 66, 71, and 82%, whereas X40 production is only inhibited by 24, 32, and 60%, respectively (Fig. 4A). To verify that the inhibitory domain within αCTF contributes to this inhibition, we determined the γ-secretase activity in the presence of both βCTF and the Sb4 substrate in which the inhibitory domain was deleted. Both βCTF and Sb4 are processed (Fig. 4B), and the Sb4 substrate did not display inhibitory activity. Taken together, these results demonstrate that αCTF acts as an inhibitor to suppress γ-secretase for βCTF processing and to reduce the production of X40. Clearly, deletion of the ASID within αCTF abolishes its inhibitory effect on γ-secretase, and thereby the existence of the ASID at the N-terminal end of αCTF is prerequisite for this negative regulation. Finally, we assessed the effect of αCTF on Notch1 cleavage (Fig. 4C). αCTF at 0.25, 0.5, and 1.0 μM inhibits γ-secretase activity for Notch1 cleavage by 7, 20, and 36%, respectively, indicating that αCTF is less effective in inhibiting Notch1 cleavage than Aβ40 production, which is consistent with our previous report using the ASID peptide (17).

**DISCUSSION**

γ-Secretase cleaves multiple substrates including APP, Notch, and other type I transmembrane proteins. γ-Secretase cleavage of substrates first requires an additional protease to remove the majority of the extracellular domain of the substrate, referred to as ectodomain shedding. However, in the processing of APP, both β- and α-secretases can shed the ectodomain by cleaving different regions of APP, which appears to be unique to APP processing. There has been a long and critical question: do β- and α-secretases play a distinctive role in the modulation of γ-secretase in the processing of APP for Aβ production? Our work suggests that α- and β-secretases are modulated by the same mechanism for regulation of γ-secretase activity through feedback inhibition. Unbalance of this system of β- and α-secretase for APP processing could lead to disease states.

Present studies have discovered an unforeseen role for α-secretase in the processing of APP, which is different from the substrate competition model. This newly defined feedback mechanism for γ-secretase regulation is proposed in Fig. 5. α-Secretase cleaves APP on the cell surface to generate the membrane-bound αCTF, which is then trafficked into intracellular compartments where it meets γ-secretase to form a low productive complex. The formation of the αCTF-γ-secretase complex ultimately leads to inhibition of γ-secretase for βCTF processing. βCTF is generated by β-secretase that predominantly resides in the late Golgi/trans-Golgi network. Active γ-secretase complex has been found in multiple compartments including endoplasmic reticulum (ER) and across plasma membrane, and increased evidence indicates that Aβ is mainly produced in the trans-Golgi network and endosomes (25). Binding modes involving interactions between the ASID of αCTF and the E site of γ-secretase could well account for the behavior of αCTF as an inhibitor and a poor substrate of γ-secretase. This notion is supported by the finding that co-expression of TACE and APP leads to a significant reduction in secreted X40 (Aβ + P3) with a concomitant accumulation of βCTF in cells. In addition, the formation of the αCTF-γ-secretase complex has been observed from the studies of γ-secretase complex isolation (26), which supports our findings. Thus, the present cellular and biochemical studies offer a new model wherein α-secretase cleaves APP to generate an endogenous inhibitor of γ-secretase that down-regulates the production of Aβ in addition to competing with β-secretase for the APP substrate (10, 11). Previously, we have shown that the ASID within βCTF interacts (cis mode of inhibition) with the Ei of γ-secretase and reduces γ-secretase activity for Aβ production (17). Both deletion and muta-
tion of the ASID within βCTF led to an increase in Aβ production (17). In fact, the Sb4 substrate has been used for the development of sensitive γ-secretase assays (27, 28) and characterization of γ-secretase (29–31). Another question that needs to be addressed is how P3 is produced at the cellular level. We propose that there is a putative factor that could interact with ASID to promote latency of its inhibitory activity. In fact, we found that the 4G8 antibody, which recognizes this sequence, is able to promote the cleavage of Sb3. Therefore, this unknown factor may possess a binding site that is similar to the antigen recognition site found in the 4G8 antibody. It could be critical to identify this factor and determine its role and specificity in the regulation of γ-secretase. In addition, it is likely that this putative factor could result from inhibition of γ-secretase by increased production of αCTF. This mechanism is also consistent with previous studies which showed that the level of sAPPα in cerebrospinal fluid was much lower in AD patients possessing the apoE4 allele compared with controls, but the amount of sAPPβ remained the same (15, 16). Moreover, decreased levels of sAPPα have been suggested as a diagnostic marker for AD (16, 32). These patient studies imply that a decrease in α-secretase activity is associated with AD. Reduction of α-secretase activity, which alleviates the negative feedback (“brake”) of γ-secretase, could be one of the major pathways in the pathogenesis of sporadic AD. Indeed, α-secretase activity is significantly reduced in the majority of AD patients (33). Thus, our model for the inhibition of γ-secretase has mechanistic implications that are critical to understanding the molecular basis for the diagnosis and pathogenesis of AD. Second, although we have demonstrated that the concentration of P3 generated in our in vitro system was not high enough to suppress γ-secretase activity, we have not excluded the possibility that in vivo the Aβ17–23 inhibitory domain of P3 might contribute to the inhibitory effects we are presently ascribing to αCTF. The possible existence of product inhibition of γ-secretase has been previously proposed by Shen and Kelleher (24).

In summary, the present studies uncover a novel function of α-secretase that is opposite to β-secretase cleavage in the regulation of γ-secretase activity. α-Secretase cleavage negatively regulates γ-secretase activity for Aβ production. This work highlights fascinating twists in the link between γ-secretase and α-secretase cleavages for APP processing and reveals an unprecedented regulatory mechanism of γ-secretase activity that could be key to our understanding of the diagnosis of and progression of AD. Furthermore, a better understanding of the interactions that regulate Aβ production is a fundamental step toward elucidating γ-secretase specificity and catalysis and designing specific inhibitors for AD therapies.

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