frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 11 December 2014
doi: 10.3389/fncel.2014.00427

The γ-secretase complex: from structure to function

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One of the most critical pathological features of Alzheimer's disease (AD) is the accumulation of β-amyloid (Aβ) peptides that form extracellular senile plaques in the brain. Aβ is derived from β-amyloid precursor protein (APP) through sequential cleavage by β- and γ-secretases. γ-secretase is a high molecular weight complex minimally composed of four components: presenilins (PS), nicastrin, anterior pharynx defective 1 (APH-1), and presenilin enhancer 2 (PEN-2). In addition to APP, γ-secretase also cleaves many other type I transmembrane (TM) protein substrates. As a crucial enzyme for Aβ production, γ-secretase is an appealing therapeutic target for AD. Here, we summarize current knowledge on the structure and function of γ-secretase, as well as recent progress in developing γ-secretase targeting drugs for AD treatment.

Keywords: γ-secretase, Alzheimer's disease, anterior pharynx defective 1, nicastrin, presenilin, presenilin enhancer 2

BACKGROUND

Alzheimer's disease (AD) is the most prevalent aging associated neurodegenerative disorder, afflicting approximately 10% of the population over age 65 and 30–50% of the population over age 85. A subset (<10%) of AD manifests as familial early-onset AD (FAD; onset in the fourth to sixth decade) and is inherited as an autosomal dominant disorder. Mutations in the genes encoding β-amyloid precursor protein (APP) and presenilins (PS1 and PS2) are causative in the majority of FAD kindred (Goate et al., 1991; Levy-Lahad et al., 1995a; Sherrington et al., 1995; Haass and De Strooper, 1999). Because the clinicopathological features of FAD are apparently indistinguishable from sporadic AD cases, great efforts have been devoted to studying these FAD linked genes and significant progress has been made to reveal mechanisms underlying AD pathogenesis.

The presence of extracellular senile plaques in the brain is a key pathological feature of AD. Senile plaques are largely comprised of variously sized Aβ peptides, where most peptides are represented by Aβ40 and the more deleterious Aβ42 species (Glenner and Wong, 1984; Masters et al., 1985; McColl et al., 2012). Aβ is produced through sequential proteolytic processing of APP by β- and γ-secretases (Haass and Selkoe, 1993; Chami and Checler, 2012). Alternatively, cell surface APP can be cleaved by α-secretase within the Aβ peptide domain to release the non-amyloidogenic soluble APPα, which has been shown to be neuroprotective (Greenfield et al., 2000). Experimental evidence from humans, animal models, and cultured cells all suggest that Aβ is the prime culprit for AD pathogenesis: excessive Aβ triggers a cascade of neurodegenerative events resulting in the formation of neuritic plaques and intra-neuronal fibrillary tangles and neuronal loss in AD (Selkoe, 1998; Greenfield et al., 2000; Golde, 2005). However, some studies suggest that the ratio of Aβ42 to Aβ40, rather than the total amount of Aβ, exhibits better correlation with the age of FAD onset (Kumar-Singh et al., 2006). Due to the importance of Aβ generation in AD pathogenesis, γ-secretase has become an important focus in AD research and has been considered as a potential therapeutic target for the treatment of AD.

SUBUNITS OF THE γ-SECRETASE COMPLEX AND THEIR ASSEMBLY

"γ-secretase" was first used to describe the proteolytic activity that cleaves APP within the transmembrane (TM) domain (Haass and Selkoe, 1993). The γ-secretase complex has since been characterized as a high molecular weight complex that consists of four essential subunits: PS (including PS1 and PS2), nicastrin, anterior pharynx defective 1 (APH-1), and presenilin enhancer 2 (PEN-2; De Strooper, 2003; Kimberly et al., 2003; Iwatsubo, 2004). Because of its complexity, almost a decade was required to identify and define all of the components of the γ-secretase complex (De Strooper, 2003).

In the early 1990s, linkage analysis was performed in several FAD families, and two AD-related loci were found on chromosome 1 and chromosome 14 (Schellenberg et al., 1992; Levy-Lahad et al., 1995b). Subsequently, independent research groups identified two homologous genes in these two loci: *PSEN1* (encoding PS1) on chromosome 14 and *PSEN2* (encoding PS2) on chromosome 1 (Levy-Lahad et al., 1995a; Rogaev et al., 1995; Sherrington et al., 1995). Until now, more than 150 different AD-causing mutations have been identified in the two PS genes, where most mutations have been found in *PSEN1* (Vetrivel et al., 2006; De Strooper et al., 2012). FAD-associated PS mutations are

directly linked to APP processing and are all seen to increase the relative abundance of the more aggregation-prone Aβ42 compared to Aβ40 (Borchelt et al., 1996, 1997; Duff et al., 1996; Scheuner et al., 1996; Citron et al., 1997; Siman et al., 2000; Flood et al., 2002; Haass et al., 2012). In addition, FAD-linked mutations in PS1 may also affect the trafficking and consequent processing of APP. For instance, FAD-linked PS1 variants can significantly reduce budding of APP-containing vesicles from both the endoplasmic reticulum (ER) and trans Golgi network (TGN), resulting in decreased delivery of APP to the cell surface and increased APP amyloidogenic processing for Aβ generation (Cai et al., 2003). PSs are multi-transmembrane proteins with an undetermined number of TM domains (Doan et al., 1996; Kim and Schekman, 2004). However a nine TM topology model with the amino-terminus in the cytosol and the carboxyl-terminus exposed to the luminal/extracellular space appears to be the most likely depiction of PS topology (Laudon et al., 2005; Oh and Turner, 2005). In general, full-length PS is inactive and rapidly removed by proteolytic degradation (Thinakaran et al., 1996; Podlisny et al., 1997; Ratovitski et al., 1997; Capell et al., 1998; Grunberg et al., 1998). Funtional PS requires endoproteolytic cleavage between TM6 and TM7 of nascently produced PS to generate a 27–28 kDa amino-terminal fragment (NTF) and a 16–17 kDa carboxyl-terminal fragment (CTF). PS NTF and CTF bind to form stable and active PS heterodimers at a 1:1 stoichiometry (Thinakaran et al., 1996, 1997; Podlisny et al., 1997; Ratovitski et al., 1997; Capell et al., 1998; Grunberg et al., 1998). PSs contain two highly conserved aspartate residues in TM6 (D257 in PS1 and D263 in PS2) and TM7 (D385 in PS1 and D366 in PS2), which are indispensable for γ-secretase activity (Wolfe et al., 1999). PS1 heterodimers can be bound by transition-state analog inhibitors of γ-secretase (Esler et al., 2000), suggesting that PSs are the crucial catalytic components of γ-secretase (Li et al., 2000; Kimberly et al., 2003); this notion is confirmed by *in vitro* assays (Ahn et al., 2010). Other PS TM domains also mediate PS function. For example, the TM1 of PS1 was shown to function as a subsite for substrate handling during the processive γ-cleavage in the hydrophilic catalytic pore together with TM 6, 7, and 9 (Takagi et al., 2010; Ohki et al., 2014).

Several studies suggested that it is impossible to overexpress functionally active PS, suggesting that additional protein components are required to form mature, stable PS heterodimers (Baumann et al., 1997; Seeger et al., 1997; Thinakaran et al., 1997; Capell et al., 1998; Yu et al., 1998; Li et al., 2000; Culvenor et al., 2004). The first PS cofactor component identified in the γ-secretase complex is nicastrin (named APH-2 in *C. elegans*), which was identified by screening for modifiers of Notch homologs *glp-1* and *lin-12* in *C. elegans* and through immunochemical purification in HEK293 cells (Goutte et al., 2000; Yu et al., 2000). Nicastrin is a 130 kDa type I TM protein that can be highly glycosylated within its ectodomain (Yu et al., 2000; Yang et al., 2002). Nicastrin is considered to be the scaffolding protein within the γ-secretase complex, and its ectodomain is proposed to bind to the free N-terminus of ectodomain-shed substrates, acting as a substrate receptor in γ-secretase (Shah et al., 2005; Dries et al., 2009).

The other two additional γ-secretase components, APH-1 and PEN-2, were identified through genetic screening in *C. elegans* (Francis et al., 2002; Goutte et al., 2002). PEN-2 spans the membrane twice, with N- and C-terminal domains facing the lumen of the ER (Crystal et al., 2003). There is only one PEN-2 homolog in mammals. Analysis of the APH-1 sequence shows that it contains seven potential TM domains, with the N-terminal domain facing the extracellular space and the C-terminal domain facing the cytosol (Fortna et al., 2004). Two APH-1 homologs, APH-1a and APH-1b have been identified in humans (Francis et al., 2002; Goutte et al., 2002), and one additional homolog APH-1c was identified in mice (Hébert et al., 2004). Mammalian APH-1a has at least two splice variants: APH-1aL and APH-1aS. Since APH1a deletion results in lethality that is not seen in APH-1b and APH-1c gene deletion in mice, different APH-1 isoforms may have different functions. Furthermore, accumulation of APP CTF in specific regions of *APH-1bc*$^{-/-}$ mouse brain (which is equivalent to *APH-1b* deficiency in humans) suggests that APH-1b might be important in the production of Aβ (Serneels et al., 2005). A recent study suggested that γ-secretase complex containing APH-1b tends to generate longer Aβ peptides than complexes containing APH-1a (Acx et al., 2014).

Several studies have demonstrated that the four components of γ-secretase cross-regulate each other coordinately. Down-regulation or deficiency of one given component typically destablizes other components and alters their trafficking/maturation (De Strooper, 2003; Iwatsubo, 2004). For example, in the absence of PS1, PEN-2 is sequestered in the ER and cannot be transported to post-ER components where the mature γ-secretase complex resides (Wang et al., 2004). PS deficiency also leads to destabilization of PEN-2 (Steiner et al., 2002; Luo et al., 2003), which is degraded via the proteasome-mediated pathway (Bergman et al., 2004; Crystal et al., 2004). On the other hand, down-regulation of PEN-2 by small interfering RNA results in an accumulation of full-length PS1 and a reduction of PS1 fragments, suggesting that PEN-2 is involved in PS1 endoproteolysis (Luo et al., 2003). Intracellular trafficking and maturation of nicastrin are also PS dependent. In the absence of PS, nicastrin fails to reach the medial Golgi compartment and becomes only partially glycosylated (Leem et al., 2002). Moreover, nicastrin deficiency reduces the levels of APH-1, PEN-2 and PS1 fragments, and affects their intracellular trafficking (Li et al., 2003a,b; Zhang et al., 2005). Similarly, in APH-1a knockout cells, the levels of nicastrin, PEN-2 and PS1 fragments are reduced (Ma et al., 2005).

The events leading to the formation of a mature γ-secretase complex start from the formation of an initial scaffolding complex composed of APH-1 and nicastrin (LaVoie et al., 2003). The proximal C-terminus of the PS holoprotein then binds to the APH1-nicastrin subcomplex by interacting with the TM domain of nicastrin (Kaether et al., 2004; Jiang et al., 2014). Following PS binding, PEN-2 is incorporated into the complex by interacting with TM4 of PS (Kim and Sisodia, 2005; Watanabe et al., 2005). At the final step, the loop domain between TM6 and TM7 of PS1 is cleaved by endoproteolysis (Fukumori et al., 2010). Alternatively, the APH-1-nicastrin subcomplex may bind directly to a cognate

PS1-PEN-2 structure to generate the mature γ-secretase complex (Shirotani et al., 2004; Capell et al., 2005). Several polar residues within APH1 TM domains have been shown to contribute to the assembly and activity of the mature γ-secretase complex (Pardossi-Piquard et al., 2009c), and have been speculated to be involved in γ-secretase substrate presentation (Chen et al., 2010). PEN-2 has also been suggested to be important for stabilizing the complex (Steiner et al., 2002; Prokop et al., 2004, 2005; St George-Hyslop and Fraser, 2012).

Several proteins have been proposed as additional γ-secretase modulatory components, which are not essential for γ-secretase activity. Proteins including CD147 (Zhou et al., 2005), TMP21 (Chen et al., 2006) and γ-secretase activating protein (GSAP; He et al., 2010) have been proposed to selectively modulate Aβ production, but do not affect Notch cleavage. CD147 is a TM glycoprotein, which interacts with all four essential γ-secretase components (Zhou et al., 2005). Downregulation of CD147 increases Aβ production, whereas overexpression of CD147 has no effect on Aβ generation (Zhou et al., 2005). However, another report suggests that CD147 modulates Aβ levels by stimulating the extracellular degradation of Aβ rather than regulating γ-secretase activity directly (Vetrivel et al., 2008). TMP21 is another protein that binds to the γ-secretase complex and regulates γ-cleavage but not ε-cleavage through its TM domain (Chen et al., 2006; Pardossi-Piquard et al., 2009a). However, another study failed to confirm the interaction between TMP21 and γ-secretase, and instead demonstrates that TMP21 influences Aβ generation through APP trafficking (Vetrivel et al., 2007). Recently, a novel GSAP was identified to selectively increase Aβ production through its interaction with both γ-secretase and the APP CTF (He et al., 2010), though interaction between GSAP and APP CTF remains controversial (Hussain et al., 2013). Although the cancer drug Imatinib was reported to also reduce Aβ levels and tau phosphorylation in an AD mouse model by modulating γ-secretase activity and GSAP levels (Chu et al., 2014), these effects could not be reproduced by other groups. Rather, additional studies found that Imatinib had no effect on blood Aβ42 levels in human cancer patients and Aβ production in rats and cell models (Hussain et al., 2013; Olsson et al., 2014).

STRUCTURAL CHARACTERIZATION OF THE γ-SECRETASE COMPLEX

The γ-secretase complex has a molecular weight of approximately 170 kDa, with an additional 30–70 kDa derived from nicastrin glycosylation (Schedin-Weiss et al., 2014), reaching a total size of about 230 kDa with 19 TMs. Structural characterization of the γ-secretase complex is very important for understanding how it recognizes and processes membrane-embedded substrates. However, clarifying details of the γ-secretase structure has gone through a long journey, mainly due to the challenge of expression and purification of an intact γ-secretase complex. The structural information of the γ-secretase complex has been primarily obtained by electron microscopy analysis with a maximum resolution of 12 Å (Lazarov et al., 2006; Ogura et al., 2006; Osenkowski et al., 2009; Renzi et al., 2011; Li et al., 2014), revealing a globular structure with several extracellular domains, three water-accessible cavities, and a potential substrate-binding surface groove in the TM region (Osenkowski et al., 2009). The first solution-state structure of human PS1 CTF was determined by nuclear magnetic resonance, demonstrating that PS1 CTF traverses the membrane three times (Sobhanifar et al., 2010), which is consistent with the widely accepted nine TM structure of PS1 (Laudon et al., 2005; Oh and Turner, 2005). Crystal structure of an archaeal PS homolog also reveals a nine TM topology, with two catalytic aspartate residues located on the cytoplasmic side of TM6 and TM7, and two potential routes for substrate entry (Li et al., 2013).

Recently a three-dimensional structure of the intact human γ-secretase complex was determined by cryo-electronmicroscopy with a resolution of 4.5 Å. The overall structural model comprises a horseshoe-shaped structure with 19 TMs and a bilobed ectodomain representing nicastrin (Lu et al., 2014). Although the resolution is still insufficient to observe atomic details, it was a pioneering step to survey the complete architecture of the γ-secretase complex. The current speculative model suggests that PS1 and PEN-2 are located to the "thick" end of the horseshoe shape, whereas APH-1 and nicastrin are located toward the "thin" end (Lu et al., 2014; Wolfe and Selkoe, 2014). Most recently, results from the same lab presented a crystal structure of nicastrin at 1.95 Å resolution, which is the first atomic-resolution structure for a γ-secretase component (Xie et al., 2014). The extracellular domain of nicastrin contains a large lobe and a small lobe. The large lobe of nicastrin, thought to be responsible for substrate recognition, associates with the small lobe through a hydrophobic pivot at the center (Xie et al., 2014). Based on this new model, nicastrin, APH-1 and PS CTF are likely to be located at the "thick" end of the horseshoe shape, whereas PEN-2 and PS NTF are located toward the "thin" end (Bolduc and Wolfe, 2014; Lu et al., 2014; Xie et al., 2014). Further work is required to elucidate structural details of other γ-secretase components at the atomic level.

BIOLOGICAL FUNCTIONS OF γ-SECRETASE

γ-secretase belongs to the family of intramembrane cleaving proteases (i-CLiPs), which includes the presenilin family of aspartyl proteases, the zinc metalloprotease site-2 protease family and the rhomboid family of serine proteases. All i-CLiPs enzymatically cleave their substrates within the plane of the lipid bilayer in a process termed regulated intramembrane proteolysis (Brown et al., 2000; Kopan and Ilagan, 2004). γ-secretase is mainly involved in intramembranous proteolysis of type I membrane proteins. It cleaves numerous functionally important proteins, such as APP (De Strooper et al., 1998), Notch (De Strooper et al., 1999), E-cadherin (Marambaud et al., 2002), ErbB4 (Ni et al., 2001), CD44 (Lammich et al., 2002), tyrosinase (Wang et al., 2006), TREM2 (Wunderlich et al., 2013) and Alcadein (Hata et al., 2012) among others, suggesting the participation of γ-secretase in a vast range of biological activities (Haapasalo and Kovacs, 2011). The best-studied γ-secretase substrates are APP for its roles in AD, and Notch for its importance in development and cell fate determination (Kopan and Ilagan, 2009; Andersson et al., 2011).

During Notch cleavage, γ-secretase releases a Notch intracellular domain (NICD) within the cytosol. NICD can translocate into the nucleus and regulate gene transcription (Kopan et al.,

1996; Schroeter et al., 1998). Notch signaling plays a critical role in short-range cell-cell communication during development, as it controls cell fate by regulating cell proliferation, survival, positioning and differentiation (Kopan and Ilagan, 2009; Andersson et al., 2011). Altered expression of Notch target genes such as hairy and enhancer of split (HES) family leads to severe developmental defects. Ablation of γ-secretase by PS1 (Donoviel et al., 1999), nicastrin (Li et al., 2003a,b) and APH-1 (Ma et al., 2005) gene deletion results in embryonic lethality in mice due to ablation of the Notch pathway. Conditional PS1 deletion in various tissues also causes defects associated with Notch pathway, such as defective T- and B-cell differentiation (Doerfler et al., 2001; Hadland et al., 2001; Qyang et al., 2004; Tournoy et al., 2004; Wong et al., 2004), bloody diarrhea as a consequence of gastrointestinal toxicity (Searfoss et al., 2003; Wong et al., 2004; van Es et al., 2005), skin and hair defects (Xia et al., 2001; Tournoy et al., 2004), and depletion of neural progenitor cells accompanied by severe morphological defects and hemorrhages in the developing brain (Kim and Shen, 2008).

APP is initially cleaved by α- or β-secretase, and the remaining membrane-bound C-terminal fragments of APP (APP αCTF and βCTF) are further cleaved by γ-secretase to generate p83 or Aβ, respectively. The p83 fragment is rapidly degraded and widely believed to have negligible function, whereas Aβ is neurotoxic (Selkoe, 2001; Zhang et al., 2011; Proctor et al., 2012; Slowik et al., 2012; Youmans et al., 2012; Chen et al., 2013; Rosén et al., 2013). In addition to releasing Aβ40 and Aβ42, γ-secretase cleavage also generates Aβ46 (ζ-site) (Zhao et al., 2004, 2007) and Aβ49 (ε-site) (Sastre et al., 2001; Weidemann et al., 2002). The existence of different Aβ species, including the shorter Aβ38 fragments suggests that γ-secretase cleaves APP in a sequential manner, first at the ε-site, followed by at the ζ-site, the γ-site, and possibly other sites (Takami et al., 2009; Okochi et al., 2013).

In addition to generating Aβ, γ-secretase cleavage of APP also generates an APP intracellular domain (AICD) within the cell. Similar to NICD, we and others have found that AICD also possess transcriptional transactivation activity and can regulate the transcription of multiple genes including APP, GSK-3b, KAI1, neprilysin, BACE1, p53, EGFR, and LRP1 (Baek et al., 2002; Kim et al., 2003; von Rotz et al., 2004; Pardossi-Piquard et al., 2005; Liu et al., 2007; Zhang et al., 2007). In addition, free AICD can induce apoptosis and may play a role in sensitizing neurons to toxic stimuli (Kinoshita et al., 2002; Giliberto et al., 2008).

ALTERNATIVE NON-PROTEOLYTIC FUNCTIONS FOR γ-SECRETASE COMPONENTS

Although PS (Donoviel et al., 1999), nicastrin (Li et al., 2003a,b), or APH-1 (Ma et al., 2005) gene deletion in mice results in lethality and abnormal embryonic phenotypes which resemble that of Notch null mice (Swiatek et al., 1994; Conlon et al., 1995; Huppert et al., 2000), specific phenotypes among different gene deletion strains are not identical, implying that each of these γ-secretase components may have its own unique physiological functions in addition to the γ-secretase activity.

PSs have been thoroughly studied for decades and has been associated with multiple functions, including calcium homeostasis, neurite outgrowth, apoptosis, autophagy, synaptic function, and tumorigenesis (Sisodia et al., 1999; Leem et al., 2002; Thinakaran and Parent, 2004; Kang et al., 2005; Lee et al., 2010; Torres et al., 2012; Bezprozvanny and Hiesinger, 2013; Eimer and Vassar, 2013; Veeraraghavalu et al., 2013; Wang et al., 2014). Several FAD mutations in PSs result in enhanced calcium release via inositol 1,4,5-trisphosphate receptors (IsnP3R) and the ryanodine receptors (RyR) receptors (Cheung et al., 2008; Hayrapetyan et al., 2008; Bezprozvanny and Hiesinger, 2013; Del Prete et al., 2014). PSs also function as passive ER calcium leak channels, and some FAD mutations in PSs disrupt the ER calcium leak function, resulting in elevated ER calcium levels and impaired store-operated calcium entry (Tu et al., 2006; Zhang et al., 2010; Bezprozvanny and Hiesinger, 2013). Moreover, autophagic/lysosomal deficits found in neurons of PS1 deficient mice indicate an essential role of PS1 in lysosomal-dependent proteolysis (Lee et al., 2010). Some studies reported that PSs could participate in neurotransmitter release and regulate synaptic scaling independent of γ-secretase activity (Zhang et al., 2009; Pratt et al., 2011).

Age-related neuronal and synaptic loss and synaptic plasticity deficits in nicastrin conditional knockout mice demonstrates essential roles of nicastrin in regulation of learning and memory and the maintenance of neuronal survival in the brain (Tabuchi et al., 2009; Lee et al., 2014). Furthermore, nicastrin is found to control cell death via Akt and p53-dependent pathways at the post-transcriptional level in a γ-secretase activity-independent manner (Pardossi-Piquard et al., 2009b). APH-1 and PEN2 are also shown to trigger an anti-apoptotic response by lowering p53-dependent control of caspase-3 (Dunys et al., 2007).

γ-SECRETASE AS A THERAPEUTIC TARGET FOR AD

γ-secretase is an attractive therapeutic target for AD due to its essential role in the generation of Aβ. Early drug discovery efforts focused on the development of γ-secretase inhibitors (GSIs). However, general inhibition of γ-secretase may potentially result in severe consequences by interfering with other physiological and developmental processes such as its involvement in proteolysis of non-AD components including Notch (Wong et al., 2004; Haapasalo and Kovacs, 2011; Imbimbo et al., 2011; Schor, 2011; Tamayev and D'Adamio, 2012). In a phase III clinical trial of the GSI semagacestat, it was found that semagacestat not only had no effect on improving cognitive status, but also was associated with more adverse events including skin cancers and infections, compared to placebo controls (Doody et al., 2013).

Therefore, the drug discovery efforts have shifted to the development of γ-secretase modulators (GSMs), which are γ-secretase targeting compounds that alter Aβ production without significantly lowering the normal physiological function of Notch and other substrates(Crump et al., 2013). A subset of nonsteroidal anti-inflammatory drugs (NSAIDs) was the first GSM compounds identified (Weggen et al., 2001). R-flurbiprofen (or Tarenflurbil), a single enantiomer of a clinically approved racemic NSAID, had showed some efficacy in a phase II clinical trial with a subgroup of patients suffering from mild AD (Wilcock et al., 2008). However, R-flurbiprofen did not show significant improvement compared to placebo controls during phase III clinical trials (Green et al., 2009). The first generation of GSM

compounds demonstrate limited pharmacological potential due to low potency and undesired neuropharmacokinetic properties, while second generation GSMs such as E2012 and EVP-0015962 show improved potency and brain availability and encouraging preclinical profiles in recent years (Oehlrich et al., 2011; Pettersson et al., 2011, 2013). Second generation GSMs can be generally divided into acid GSMs, non-acid GSMs and natural product derived GSMs (Crump et al., 2013; Golde et al., 2013). Acid GSMs, including GSM-1 and its analogs (GSM-2 and GSM-10h) and EVP-0015962 usually reduce Aβ42 and increase Aβ38 levels (Page et al., 2008; Hawkins et al., 2011; Mitani et al., 2012; Rogers et al., 2012). E2012, the first non-acid GSM to enter clinical development, lowers Aβ42 and Aβ40 and raises Aβ37 and Aβ38 levels (Portelius et al., 2010; Borgegard et al., 2012; Crump et al., 2013). Recently identified natural product derived GSMs appear to be unusual as they decrease both Aβ42 and Aβ38 (Hubbs et al., 2012; Loureiro et al., 2013).

CONCLUDING REMARKS

The γ-secretase complex plays crucial roles in various physiological processes. Because of the importance of γ-secretase in Aβ generation, γ-secretase has been targeted for AD drug development, but with little success so far due to the complexity of its structural organization and the varied nature of its multiple substrates. A better understanding of the structure-function relationship of γ-secretase will help in developing modulators which limit cleavage of other important physiological γ-secretase substrates for use in AD therapy.

ACKNOWLEDGMENTS

We thank Timothy Huang for proofreading this manuscript. This work was supported by grants from National Natural Science Foundation of China (81225008, 81161120496, 91332112, 91332114 and 81000540), Natural Science Foundation of Fujian Province of China (2010J01235), and Fundamental Research Funds for the Central Universities of China.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 21 November 2014; accepted: 27 November 2014; published online: 11 December 2014.

*Citation: Zhang X, Li Y, Xu H and Zhang Y (2014) The γ-secretase complex: from structure to function. Front. Cell. Neurosci. **8**:427. doi: 10.3389/fncel.2014.00427*

This article was submitted to the journal Frontiers in Cellular Neuroscience.