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The γ-Secretase Complex: From Discovery to Therapeutic Target

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ABSTRACT

γ-Secretase is an intricate intramembrane aspartyl protease that cleaves within the transmembrane domain of ~150 substrates and considered the 'proteasome of membrane'. This enzyme consists of four different subunits, with presenilin being the catalytic subunit. This review provides a brief overview of γ-secretase as a proteolytic enzyme, from its biochemistry and biology to its roles in disease and potential as a therapeutic target. A detailed discussion on the discovery and structure of γ-secretase is followed by a survey of its substrates, including the most studied amyloid precursor protein and Notch1 receptor, and description of substrate processing and sequence specificity. The role of γ-secretase in human biology and pathology is also detailed, with a particular focus on Alzheimer's disease (AD), in which the pathogenicity of the γ-secretase product amyloid-β peptide is still a matter of controversy. Lastly, the potential of γ-secretase inhibitors and modulators for the treatment of AD and other diseases is considered.

KEYWORDS: Alzheimer's disease, protease, presenilin, inhibitors, modulators

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INTRAMEMBRANE PROTEASES

Intramembrane proteases (IMPs), also known as intramembrane-cleaving proteases (I-CLiPs), are transmembrane enzymes, with their active site located within the hydrophobic environment of lipid bilayer.[1] IMPs show a range of substrate specificities, but all cleave within the transmembrane domains of their substrates. Although mechanistically similar to water-soluble proteases, IMPs are not evolutionarily related to these classical proteases. Moreover, the catalytic rates of IMPs are extremely slow.[2,3,4,5] IMPs cleave membrane protein substrates within their transmembrane regions by a process called as Regulated Intramembrane Proteolysis (RIP), which is conserved from bacteria to humans.[6,7] RIP involves two regulated aspects, the first being ectodomain shedding of the substrates prior to intramembrane proteolysis by 'sheddases' (with the exception of rhomboid serine protease) and second being transport/trafficking of involved enzymes or their substrates.[8,9] IMPs create an environment within the lipid bilayer that is suitable for water and hydrophilic residues to conduct hydrolysis of their substrates.[10] These enzymes are essential in biology,[10] and IMP-mediated cleavage events are often signaling mechanisms, such as in the Notch signaling pathway and EGF (Epidermal Growth Factor) pathway. Impaired functioning of IMPs occurs in various pathological conditions, including Alzheimer's Disease (AD), cancer, Parkinson's disease (PD), diabetes, and more.[11,12] Many IMP members have been identified since their first discovery in 1997.[13] Based on the catalytic mechanism, the four classes of known IMPs are metalloproteases such as Site-2 protease (S2P), rhomboid serine proteases, glutamyl IMPs such as Rce1, and aspartic IMPs such as γ-secretase and signal peptide peptidase.[10,14,15,16,17,18] This review discusses in detail a founding member of the IMP family, γ-secretase, along with its many substrates, roles in human diseases, and therapeutic potential.

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DISCOVERY AND COMPONENTS OF THE γ-SECRETASE COMPLEX

Plaque deposits of the 4-kDa amyloid β-peptide (Aβ) are found in the brains of Alzheimer's disease (AD) patients. This fragment is generated from the amyloid precursor protein (APP) through successive cleavage by two proteases: β-secretase and γ-secretase.[19] γ-Secretase is the most biochemically complicated of the IMPs.[20] The proteolytic activity that produces Aβ from APP was first described as "γ-secretase" in relation to AD more than three decades ago;[21,22] however, the enzyme and its components were not fully identified until a decade later.[23] Around the same time, missense mutations associated with dominantly inherited familial AD (FAD) were found in APP[24,25] as well as in presenilin-1 and -2 (PS1 and PS2).[26,27] PS1 activity was soon linked to Aβ as FAD PS1 mutations were shown to alter its production.[28,29] The discovery that PS1 deficiency led to substantial reduction of Aβ production, suggested that PS1 mediates most proteolytic cleavage of APP, with the remainder cleaved by PS2.[30,31,32] Presenilin FAD mutations were found to elevate the proportion of the Aβ42 variant relative to Aβ40.[28,33,34,35,36] All these observations regarding presenilin were made in the context of γ-secretase. Cell-based assays for Aβ production were used to test peptidomimetics as inhibitors of γ-secretase, and these inhibitors suggested that the enzyme is an aspartyl protease.[37] It was then discovered[38] and further confirmed[39,40] that two conserved transmembrane aspartates in presenilins are critical for γ-secretase cleavage activity. Transition-state analog peptidomimetic inhibitors of γ-secretase were subsequently found to bind with the presenilin active site.[41,42] Simultaneously, it was discovered that presenilin-dependent γ-secretase activity that is responsible for APP cleavage is also involved in and crucial for transmembrane cleavage of the Notch1 receptor to release the Notch intracellular domain (NICD), a seconder messenger for cell signaling.[43,44,45,46,47,48,49] All these discoveries

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collectively identified presenilin as a membrane-embedded aspartyl protease, the catalytic component of γ-secretase.

Presenilin is expressed in the endoplasmic reticulum (ER) and undergoes proteolysis to form N-terminal and C-terminal fragments (NTF and CTF, respectively).[50,51,52] These fragments were found to remain associated within a higher molecular weight complex,[53,54,55] suggesting that presenilin is a part of a larger complex. When the two conserved transmembrane aspartates were discovered to be essential for γ-secretase activity, they were also found to be required for presenilin cleavage into NTF and CTF, indicating presenilin is a zymogen that cleaves itself into its active form.[38] Later, three additional γ-secretase components were identified through biochemical and genetic studies as nicastrin (NCT),[56,57] anterior pharynx defective 1 (APH-1)[58,59,60] and presenilin enhancer 2 (PEN-2).[61,62] All four membrane protein components assemble together in 1:1:1:1 stoichiometry, with presenilin undergoing proteolysis to NTF and CTF to form the proteolytically active γ-secretase complex.[63,64,65,66] No additional proteins were found to be stably connected with the complex. Enzyme activity is regulated mainly by its primary components; however, additional regulation may be provided by lipid composition and by other associated (non-essential) protein factors that may modulate the enzyme complex.[67,68] The complex of γ-secretase enzyme is depicted in Fig. 1

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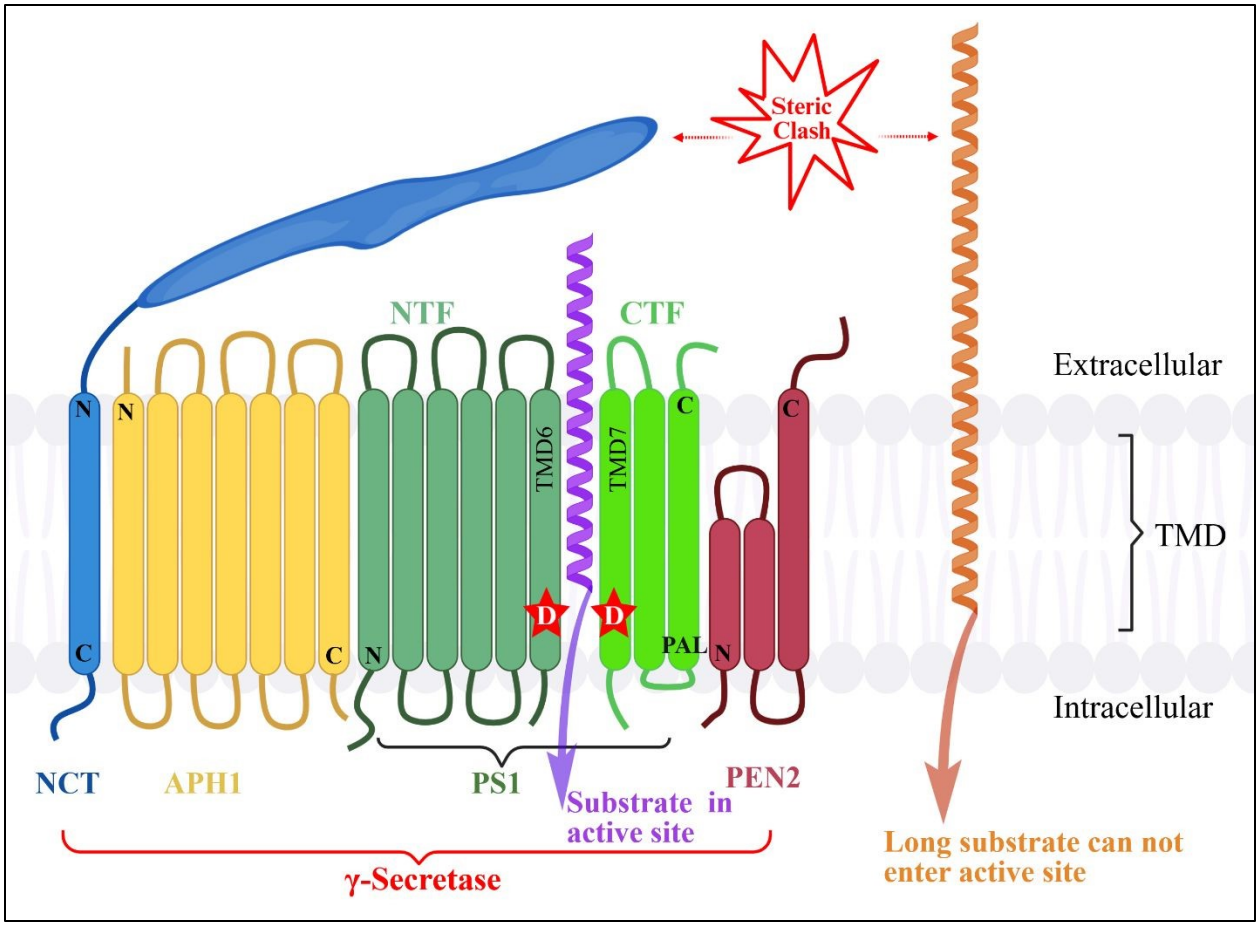

Figure 1: The γ-secretase complex and substrate interaction. A γ-secretase complex containing all four components NCT (blue), APH-1 (yellow), Pen-2 (Red) and catalytic PS1 NTF and CTF (dark and light green) is shown. Catalytic aspartates are marked by D in TM6 and TM7 of PS1. The substrate entry in the active site is guided by NCT in extracellular region. The short substrate (purple) is entered in the active site while the longer substrate (orange) cannot enter the active site because of the steric clash between the long ectodomain of the substrate and NCT extracellularly. NCT: nicastrin, APH1: anterior pharynx defective-1, PEN2: presenilin enhancer 2, PS1: presenilin1, PAL: $P_{433}A_{434}L_{435}$ motif, N: N-terminal, C: C-terminal, TMD: transmembrane domain, NTF: N-terminal fragment, CTF: C-terminal fragment.

Presenilin has nine transmembrane domains (TMDs),[69] with the two catalytic aspartates (D257 and D385 in PS1) residing in TMD 6 and 7.[38] The C-terminally conserved '$P_{433}A_{434}L_{435}$' motif in TMD9 of PS1 is an important part of the active site of PS1 and also for proteolytic activity of the enzyme.[70,71,72] The PAL motif is essential for PS1 endoproteolysis[70] and contributes towards proper active site conformation of the enzyme.[71] Another motif, GxGD, which contains one of the

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two catalytic aspartates (D385), is critical for substrate specificity, selectivity and proteolytic activity of γ-secretase.[73,74]

The other components of γ-secretase are essential in forming the mature enzyme complex.[75] NCT has only one TMD, a small intracellular and a large extracellular domain.[56] It plays a role in substrate recognition[76] and their selective recruitment through steric hindrance.[77] In contrast, another report says that NCT helps stabilize the enzyme complex but is not essential for substrate recognition.[78] NCT is essential for APP processing and is critical but not absolutely required for Notch processing.[79] The role of APH-1 is apparently to stabilize the enzyme complex, serving as a scaffold for assembly, while PEN-2 is essential for endoproteolytic processing of PS1.[23,65,80] APH-1 has seven TMDs, with the C-terminal end facing cytosol and N-terminal end facing extracellular space,[81] and is reported as not being absolutely required for processing of APP or Notch substrates.[79] PEN-2, was predicted to have only two TMDs, with both the C- and N-terminal ends facing the extracellular region and a connecting loop in the cytosol.[82] PEN-2 is also known to be involved in maturation and stability of the enzyme complex.[83,84] All four components need to be assembled in correct order for the enzyme to be fully active. In endoplasmic reticulum, NCT and APH-1 first bind together, which allows binding of full-length PS to the complex followed by PEN-2. TMD4 of PS1 interacts with PEN2 in complex formation,[85] which then triggers autoproteolysis of PS1 into NTF and CTF. Furthermore, the TMD1 sequence of PEN2 (specifically the proximal 2/3rd part) is essential for autoproteolysis of PS1.[86] The complex then travels to the Golgi for glycosylation,[75,87] and further traffics through the secretory pathway to the cell surface and to endosomes and lysosomes. The sequential assembly of all the components to form active enzyme γ-secretase complex is shown in Fig. 2

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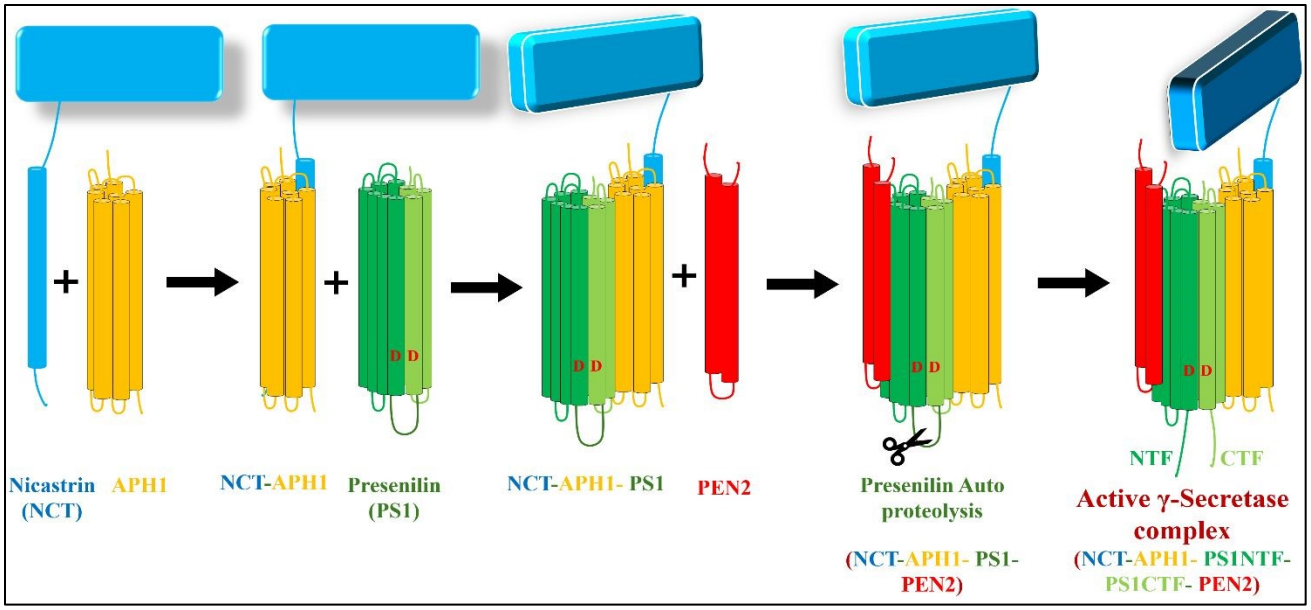

Figure 2: Sequential assembly of γ-secretase components into full active complex All four γ-secretase components of the enzyme assemble in sequential manner to form a full complex, which is activated by autoproteolysis. First nicastrin (NCT blue) and APH-1 (yellow) come together to form NCT-APH1 complex. Presenilin1 (PS1 dark and light green) associates itself with the NCT-APH1 complex forming NCT-APH1-PS1 complex. PEN-2(Red) then interacts with TMD4 of PS1 in the complex forming NCT-APH1-PS1-PEN2 complex. The interaction of PEN-2 and PS1 causes autoproteolysis of PS1 in NTF and CTF, generating fully activated γ-secretase complex. Catalytic aspartates are marked by D in TM6 and TM7 of PS1.

STRUCTURE OF THE γ-SECRETASE COMPLEX

Cryo-electron microscopy (cryo-EM) images of γ-secretase have provided detailed insight into the structure of the protease complex and interaction with substrates over the last decade. The first high-resolution cryo-EM structure of intact γ-secretase, determined at 4.5 Å resolution,[88] revealed 19 TMDs arranged in a horseshoe shape. The active site is located on the convex side of this horseshoe-shaped TMD complex, with the NCT ectodomain positioned above the PS active site, serving as a gatekeeper to allow entry of only substrates with short ectodomains (Fig. 2).[77,88,89,90] Structural organization of the enzyme subunits and their TMDs that were proposed based on biochemical studies were confirmed by these cryo-EM studies. PS1 is central in the complex with

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its NTF attached to PEN2 and CTF interacting with APH1, which interacts with the lone TM of NCT.[91] Cryo-EM studies further clarified that γ-secretase has 20 TMD instead of 19. Specifically, PEN2 contains three TMD in contrast to previously proposed two.[82] TM2 of PEN2 only goes into the membrane halfway through and turns back into the cytoplasm, localizing its N-terminus into the cytoplasm whereas the C-terminus is localized towards the extracellular side.[91] The poorly resolved TMD2 of PS1 suggested that TMD2 is flexible and involved in lateral entry of substrate TMD in the active site. Subsequently, substrate binding is proposed to cause conformational changes in the enzyme that activates the two catalytic aspartates by bringing them into proximity.[87] Additionally, part of TMD6 was also unresolved, presumably because of flexibility, suggesting that, together with TMD2, it likely regulates substrate entry.[92]

Subsequent cryo-EM structures of γ-secretase bound to substrates APP and Notch, at 2.6-2.7 Å resolution, provided further details of enzyme interactions with specific substrates.[93,94] These studies revealed that substrates acquire a hybrid α-helical/β-sheet structure that exposes the cleavage site in the active site and support a substrate-helix unwinding model in the active site of γ-secretase for their cleavage. Furthermore, the lateral diffusion model for substrate entry and involvement of TMD2 and part of TMD6 of PS1 was also confirmed, as these two regions were well resolved upon substrate binding.

The γ-secretase structure bound to the dipeptide analog inhibitor DAPT (N-[N-(3,5-difluoro-phenacetyl)-L-alanyl]-S-phenylglycine tert-butyl ester) showed the inhibitor binding close to the active site. The structure also suggested that DAPT binding induces a conformational change in PS1 which does not allow entry of substrate, hence inhibiting enzyme activity.[95] The recent structures of the protease bound to three other inhibitors (semagacestat, avagacestat, and the peptidomimetic transition state analog L685,458) showed that all three γ-secretase inhibitors

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(GSIs) interact with the same site on PS1 occupied by the APP or Notch β-strands in and around the active site, hence inhibiting substrate binding.[96] In contrast, the structure of γ-secretase bound to E2012 revealed that this γ-secretase modulator (GSM) binds extracellularly to an allosteric site on the protease complex.[96]

Computational molecular dynamics (MD) studies have also provided useful insights into the mechanism and dynamics of substrate-enzyme interaction, substrate cleavage, conformational changes, enzyme inhibition and modulation.[97,98] For example, combining MD and biochemical studies helped understand APP substrate processing by γ-secretase[99] and develop a model of γ-secretase-Notch complexes for Notch wild-type and mutant cleavage.[100] In the latter study, an incorrect registry of Notch1 binding was identified in the cryo-EM structure, resolving discrepancies with biochemical results by systematic replacement of bound APP residues with corresponding Notch residues. The model developed through this "replacement method" was highly consistent with biochemical results.[100]

VARIOUS SUBSTRATES OF γ-SECRETASE

γ-Secretase is currently known to cleave more than 145 substrates, including APP and the Notch1 receptor.[101] The products generated from these cleavage events have varying functions. Most of the substrates are type I integral membrane proteins[90] with long ectodomains that require shedding before γ-secretase processing.[102] γ-Secretase cleavage of substrates is not dependent on particular consensus sequence; however, short extracellular domains are required for higher cleavage efficiency (Fig. 1).[77,103] Because of different isoforms for PS and APH found in human, multiple enzyme complex subtypes have been identified.[67] Based on their locations and expression levels in the human body, different substrates may be cleaved by different complexes.[101]

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Although some common features of most γ-secretase substrates are noted,[68] the enzyme promiscuously cleaves various substrates, and there is no known consensus sequence.[104] Moreover, in cases of substrates such as Notch,[47] APP,[105] and CD44,[106] proteolytic processing by γ-secretase generates multiple products with varying C-terminal ends. Hence, the enzyme has been dubbed as 'the proteasome of the membrane', cleaving many substrates within their TMDs and playing critical roles in biology and medicine.[107] The enzyme has a substrate-binding exosite site at the PS1 NTF/CTF interface that is distinct from but proximal to the active site.[108] The first (endoproteolytic or ε) cleavage occurs near the cytosolic interface of the membrane, releasing the fragment called intracellular domain (ICD) into the cytosol (Fig. 3). Although a wide range of substrates are known for the enzyme, specific cleavage sites have been identified for very few. In this review, we will discuss two well-studied γ-secretase substrates, APP and Notch1, in detail and briefly touch upon other substrates, followed by discussion of a substrate cleavage model and sequence specificity.

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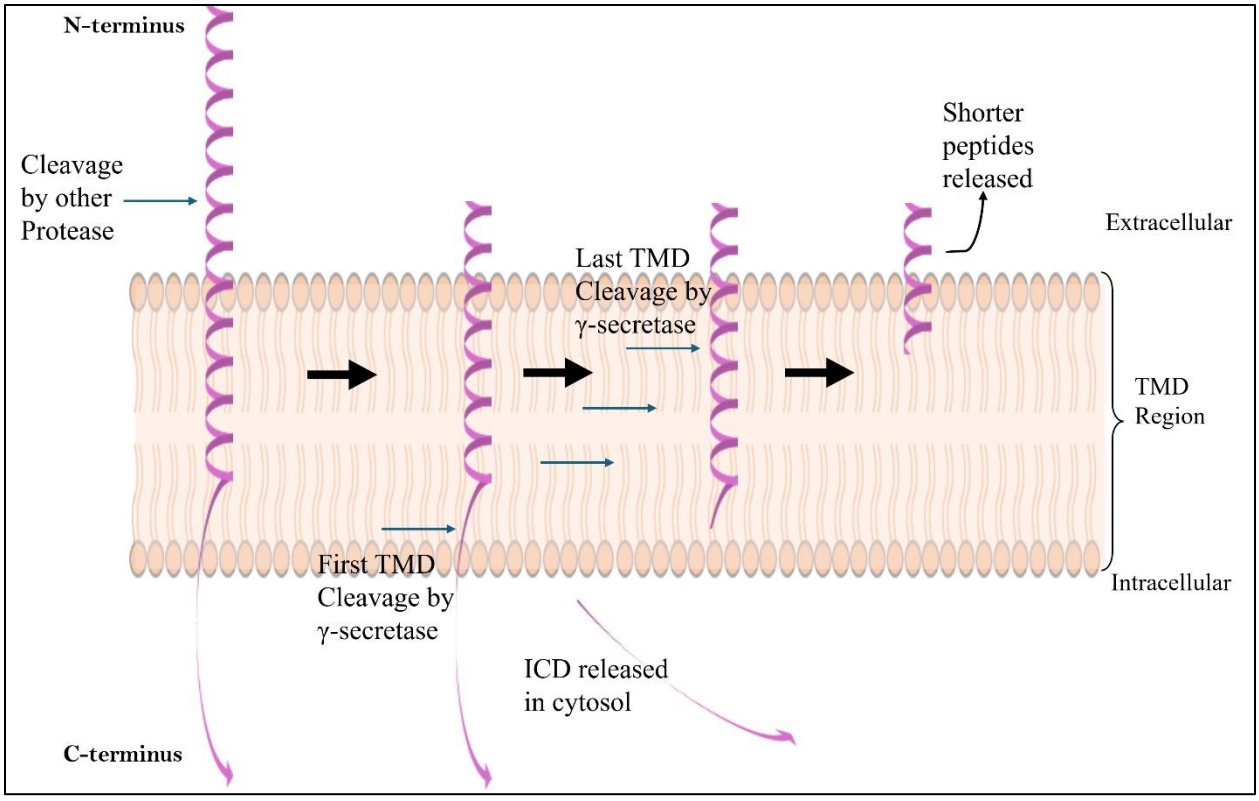

Figure 3: General process of substrate cleavage by γ-secretase. The type-1 transmembrane domain (TMD) substrate with long ectodomain is first cleaved by protease close to the surface of TMD generating a shorter membrane-bound fragment that enters the active site of the enzyme. γ-Secretase then cleaves at ε-site first near cytosolic end releases C-terminal cleavage product (intracellular domain: ICD) in cytosol. Then, the remaining membrane-bound fragment is trimmed further down and released in the extracellular space as a N-terminal product.

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Amyloid precursor protein (APP)

Amyloid precursor protein (APP) is a single-pass conserved type I integral membrane protein. The *APP* gene is located on chromosome 21, and tissue-dependent alternate splicing leads to expression of three major isoforms, of which APP695 is almost exclusively found in the brain.[109,110] Two other homologous APP-like proteins (APLP-1 and APLP-2) are known; however, they do differ in sequence in the Aβ region.[111] The proteolysis of full-length APP involves multiple cleavages by proteases and can occur by two different pathways: amyloidogenic and non-amyloidogenic, as shown in Fig. 4.

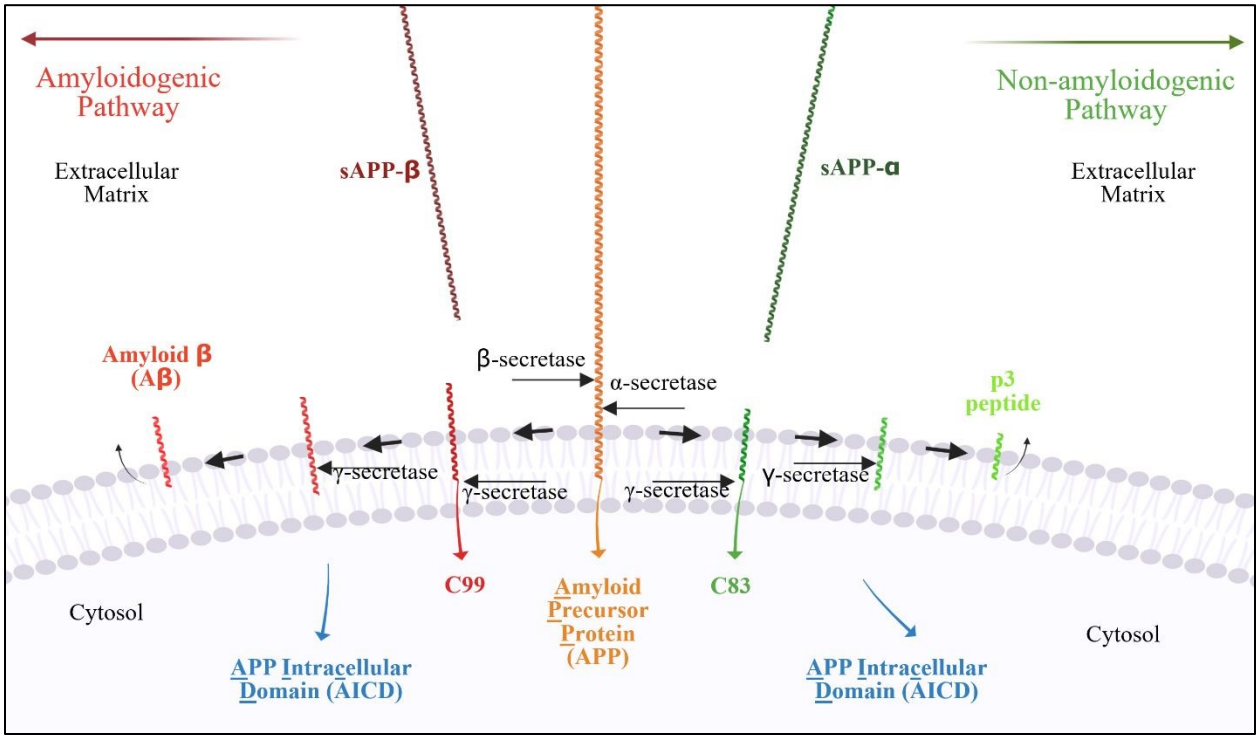

Figure 4: The proteolysis of full-length APP by amyloidogenic (red) and non-amyloidogenic (green) pathways. The long ectodomain of APP is cleaved by either secretase shedding ectodomain extracellularly. The membrane bound C99 is then cleaved by γ-secretase to release AICD fragment in cytosol. Membrane-bound longer Aβ fragments are then further trimmed down by γ-secretase to release shorter fragments in extracellular space.

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The amyloidogenic pathway (Fig. 4) starts with the juxtamembrane cleavage of full-length APP by a pepsin-like aspartyl protease, β-secretase (also known as BACE1: β-site APP-cleaving enzyme), releasing the soluble extracellular domain called sAPP-β and leaving behind a membrane-anchored stub containing 99 amino acids called β-carboxy-terminal fragment (β-CTF or APP-C99).[112–117] C99 then undergoes cleavage by γ-secretase within its single TMD generating amyloid β-peptide (Aβ) and APP intracellular domain (AICD). Recent reviews suggest that C99, rather than Aβ, may be a culprit that accumulates in AD and acts as an early pathogenic trigger.[118,119] The γ-secretase processing of C99 is discussed in further detail later.

Alternatively, in the non-amyloidogenic pathway (Fig. 4), the full-length APP is cleaved within the extracellular region of the Aβ sequence by α-secretases, ADAM-like metalloproteases, to release a longer soluble ectodomain called sAPP-α and leaving behind a membrane-bound fragment of 83 amino acids called C83 (or α-CTF).[117,120] The latter is further cleaved by γ-secretase to generate AICD and N-terminally truncated Aβ-like peptide dubbed p3.[117] The α-secretase cleavage of APP is the most abundant pathway in cells.[120] APP and its various proteolytically derived fragments have been proposed to have different biological roles, which are reviewed in detail elsewhere.[109,110,111]

Notch receptor family

Notch receptors are another type I integral membrane protein that are arguably the most important substrates of γ-secretase. The *Notch* gene was first identified in *Drosophila*, encoding for a 300 kDa protein. In mammals, four Notch isoforms Notch1-4 are present and structurally similar to *Drosophila* Notch and *C. elegans* lin-12 and glp-1.[121] These evolutionarily conserved

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cell-surface receptors are crucial to development and health of all metazoans, as they are involved in cell proliferation, homeostasis and damage repair.[122]

Aberrant Notch signaling can cause various cancers and other diseases.[123,124] Notch protein has a large extracellular domain which undergoes post-translational modifications (PTM), including glycosylation and S1 cleavage. In the secretory pathway, during maturation, a furin-like convertase cleaves full-length Notch at the S1 site, generating a heterodimeric receptor that translocates to the cell surface. At the cell surface, interaction with cognate ligands (Delta and Jagged) on an adjacent cell triggers conformational changes that make the Notch1 heterodimer accessible to ADAM10 metalloprotease for cleavage at the extracellular juxtamembrane S2 site, releasing the ectodomain.[125,126] This cleavage leaves behind the membrane-bound Notch extracellular truncation (NEXT).[127] NEXT is then cleaved at the S3 site (between G1743 and V1744 for murine, G1753 and V1754 for human) within the membrane near the cytosolic side by γ-secretase, releasing the Notch intracellular domain (NICD)[43,44] into the cytosol. The remnant membrane-anchored stub of Notch, known as Nβ, is further cleaved within its TMD by γ-secretase at S4 sites releasing shorter, secreted Nβ species.[47,128] The NICD translocates to the nucleus and initiates gene expression after interaction with the DNA-binding transcription factor CSL.[44,129] Hence, the S3 cleavage by γ-secretase is an essential step in this crucial signaling pathway. The proteolysis of Notch1 by γ-secretase is shown in Fig. 5.

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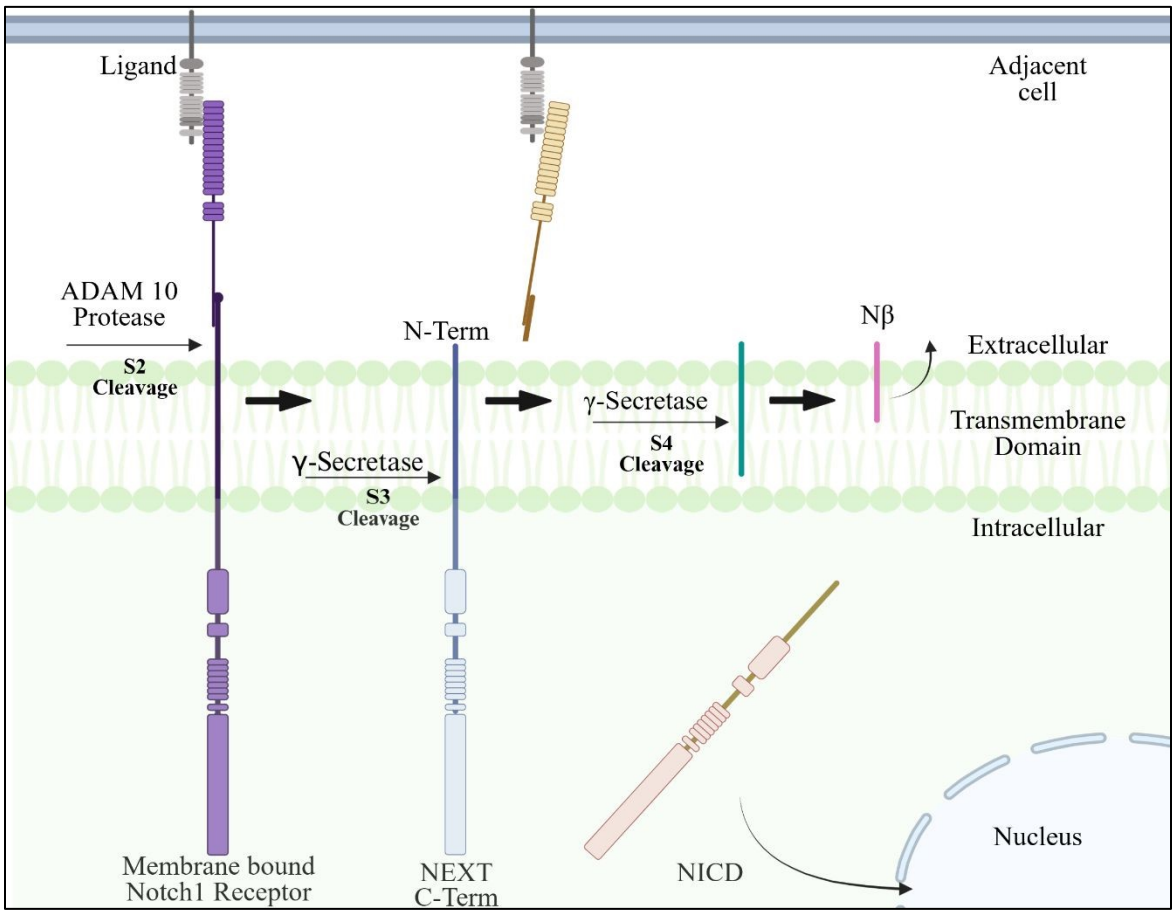

Figure 5: The process of Notch1 proteolysis by γ-secretase. Membrane bound full-length Notch1 receptor (Purple) is cleaved by ADAM 10 protease after binding with the ligand from adjacent cell generating NEXT (Notch Extracellular Truncation). NEXT is then cleaved by γ-secretase at S3 cleavage site releasing NICD (Notch Intracellular Domain) that traverse to nucleus for gene expression. The remaining membrane bound Notchβ (Nβ) is further trimmed by γ-secretase to release smaller Nβs in extracellular space.

Other substrates

B cell maturation antigen (BCMA), a cell surface receptor, is a short γ-secretase substrate, that does not need ectodomain shedding before γ-secretase cleavage and is involved in regulation of plasma cell survival by interaction with its ligands.[130] Another γ-secretase substrate, Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) is a cell-surface receptor involved in signaling pathways that regulate cytokine secretion and phagocytosis.[131] The soluble form of TREM2

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released after ADAM-like proteolytic cleavage produces inflammatory cytokines and is involved in survival of microglia.[132] Furthermore, many cytokines and other immune receptors including TNFR1, IL-1R1, IL-1R2, IL6R, CX3CL1 are also important γ-secretase substrates.[133]

CD44, a γ-secretase substrate, is an adhesion protein that is expressed in most cells and involved in intracellular signal transduction.[134] CD44 is physiologically important in hematopoiesis, immune system maintenance, wound healing as well as involved in pathological conditions such as cancer.[134,135] Another important class of cell-cell adhesion proteins, cadherins, are substrates for γ-secretase.[136,137] These Ca^{2+}-dependent proteins mediate cell adhesion via adherens junctions (AJ) and bind to intracellular components such as catenins in the cytoplasmic region.[138,139] Two more classes of proteins that act as synaptic cell-adhesion molecules are pre-synaptic neurexin (NRX) and its primary post-synaptic partner neuroligin (NLG), which are essential in synapse formation and function.[140,141] Their function at synapses is regulated by γ-secretase processing.[142,143,144,145] γ-Secretase substrates neuregulin (NRG) and its receptor epidermal growth factor receptor (ErbB) are involved in development and functioning of the nervous system.[146] γ-Secretase cleavage of cell surface protein family receptor tyrosine kinases (RTKs) generates RTK ICDs which regulate their signaling pathways. These pathways ultimately activate gene transcription and modulate cell activity. Out of 55 known human RTKs, 27 RTKs have so far been identified as substrates for γ-secretase.[147]

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SUBSTRATE PROCESSIVE PROTEOLYSIS AND SEQUENCE SPECIFICITY

Substrate trimming and the three pocket model

Substrate trimming by γ-secretase is discussed in the context of APP processing, which has been extensively studied and is shown in Fig. 6. APP proteolysis by γ-secretase is a complex process.[148] Initially, APP C99 undergoes endoproteolysis at the ε-site after residues Leu49 or Thr48 generating Aβ49 or Aβ48, respectively, with release of the corresponding APP intracellular domain (AICD), AICD 50-99 or AICD 49-99.[149,150,151] Total Aβ and AICD are generated in equimolar proportion.[152] γ-Secretase then cleaves Aβ49 and Aβ48 in three amino acid increments through its carboxypeptidase activity, producing tripeptide co-products as well as Aβ40 or Aβ42.[105,153,154,155] Hence, two main pathways that generate Aβ40 or Aβ42 are: C99→Aβ49→ Aβ46→ Aβ43→Aβ40 and C99→Aβ48→ Aβ45→ Aβ42 respectively (Fig. 6). Aβ40 can be further trimmed to Aβ37, while Aβ42 is trimmed to Aβ38, generating a tetrapeptide co-product.[155,156] A clear reason behind generation of tetrapeptide is unknown, however, it is thought that the enzyme prefers cleavage between less crowded G38-V39 bond instead of more crowded V39-V40 bond to generate Aβ38 and tetrapeptide.[155] The longer forms of Aβ peptides (Aβ45-Aβ49) remain bound to the membrane until further processed by γ-secretase and not found extracellularly.[105] Additional minor alternative pathways have also been observed, ultimately yielding shorter, secreted forms of Aβ.[157] Notch1 is very likely processed similarly by the enzyme.[47,128]

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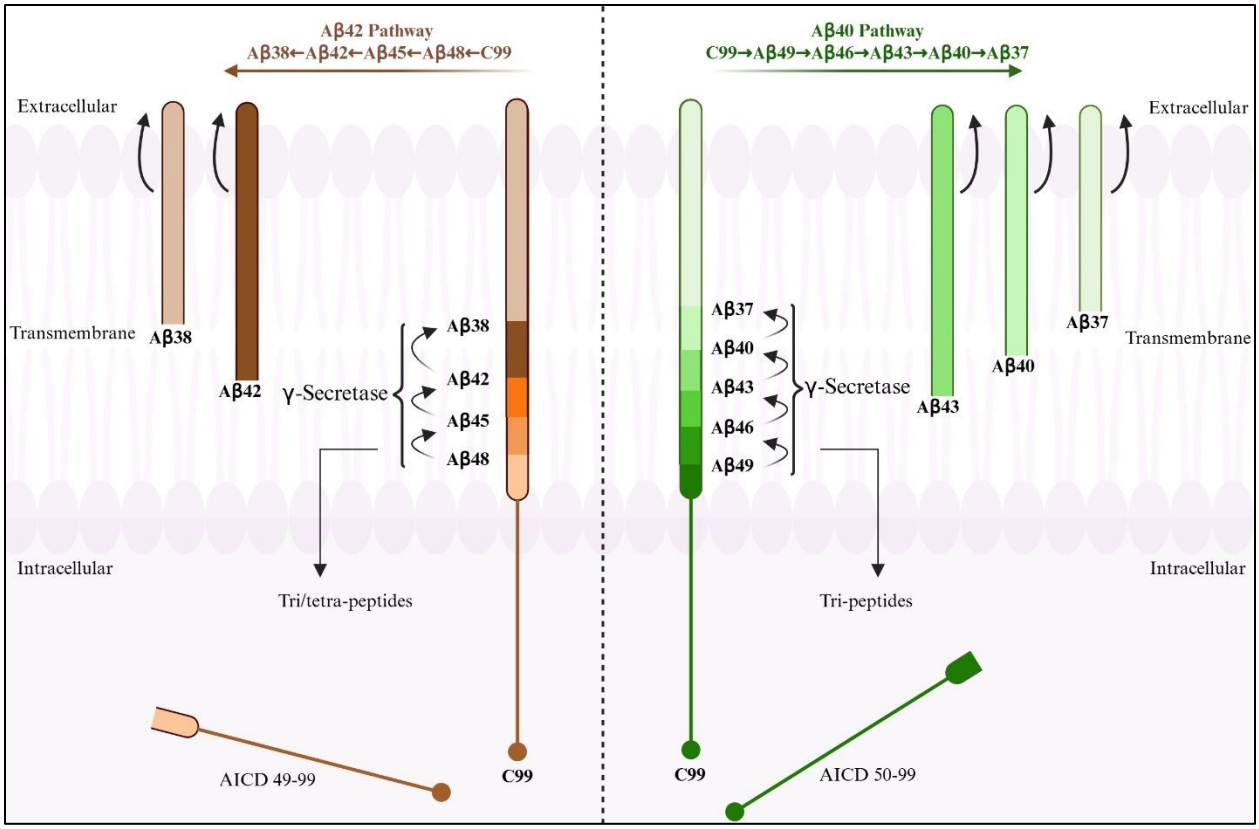

Figure 6: Processive proteolysis of C99 by γ-secretase along Aβ40 and Aβ42 pathway. Two main pathways that generate Aβ40 (C99→Aβ49→ Aβ46→ Aβ43→Aβ40) and or Aβ42 (C99→Aβ48→ Aβ45→ Aβ42) are shown. Also, corresponding AICD species (50-99) and (49-99) are also shown. The longer Aβ45-49 remain bound to the enzyme in the membrane until further trimmed by the enzyme. The shorter Aβ are released in extracellular space, while tri/ tetrapeptides are released in cytoplasm.

Early molecular probes for γ-secretase, peptidomimetic transition-state analogue (TSA) inhibitors, were initially used to probe the active site binding pockets of the enzyme.[87] These probes suggested three pockets in the enzyme active site (S1', S2', and S3') that could accommodate three substrate residues (P1', P2' and P3'), as adding a P4' residue to the TSAs did not change inhibitor potency and removing the P3' residues substantially reduced it. These probes further suggested that the S2' pocket is smaller than the other two pockets.[158,159] A substrate mutagenesis study further confirmed the "three-pocket model" of the active site, which dictates processive APP carboxypeptidase cleavage to generate tripeptide products.[160] The three pockets

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in the active site likely stabilize the unwound substrate and hence its availability for cleavage in a tripeptide pattern (Fig. 7).

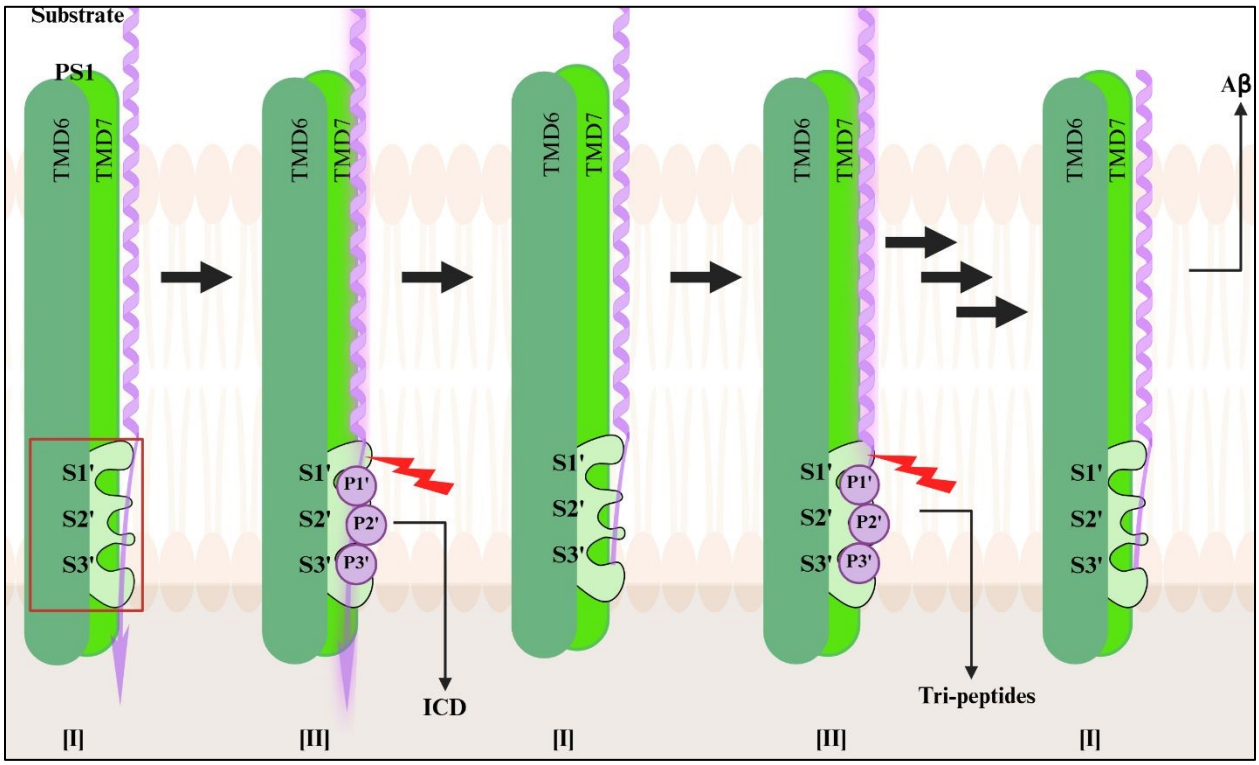

Figure 7: Substrate processing by three pocket model of γ-secretase. Three active site pockets (S1', S2' and S3') of γ-secretase are shown, with S2' being smaller than the other two. The red rectangle represents active site of the enzyme. The substrate (purple) enters in the active site [I] and unwinds to bind in the active site. The P1', P2' and P3' residues of the substrate occupy the three pockets of enzyme (purple circles) generating transition state [II] poised for cleavage by the enzyme. The process continues until tripeptides and Aβs are released intra and extracellularly respectively.

Substrate sequence specificity

As mentioned earlier, γ-secretase has no sequence specificity for cleavage of its substrates. Analysis of cleavage sites revealed that Val and Leu are favored amino acids for residues P1 (an immediate N-terminal amino acid from cleavage site) and P1'(an immediate C-terminal amino acid from cleavage site), respectively.[161] The only sequence specificity rule known for γ-secretase cleavage is that bulky amino acids such as phenylalanine (Phe) are not tolerated at the P2' position

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with respect to any cleavage event in APP.[160] The series of TSA inhibitors with Phe at the P2' position showed dramatic loss of potency.[159] The smaller S2' pocket in the enzyme active site clashes with the bulkier side chains of Phe and hence cannot bind in the active site for cleavage. However, this rule was established only for APP and its cleavage along both Aβ40- and Aβ42-producing pathways. Very recently this rule was tested for other γ-secretase substrates such as Notch1, neuregulin-1 (NRG1) and E-Cadherin (CDH1).[128,162] By installing Phe at the P2' site respective to ε-cleavage, shifts in the cleavage site were observed, indicating that this rule also applies to these other substrates. Since there are more than 145 substrates for the enzyme, further investigation is needed to determine if this rule is specific to only a few substrates or is a general phenomenon applicable to a broad range of the substrates. This phenylalanine specificity rule is depicted in Fig. 8, where the active site of presenilin is shown. The S2' pocket is smaller than other two pockets and cannot accommodate phenylalanine, hence a shift in the cleavage site is observed.

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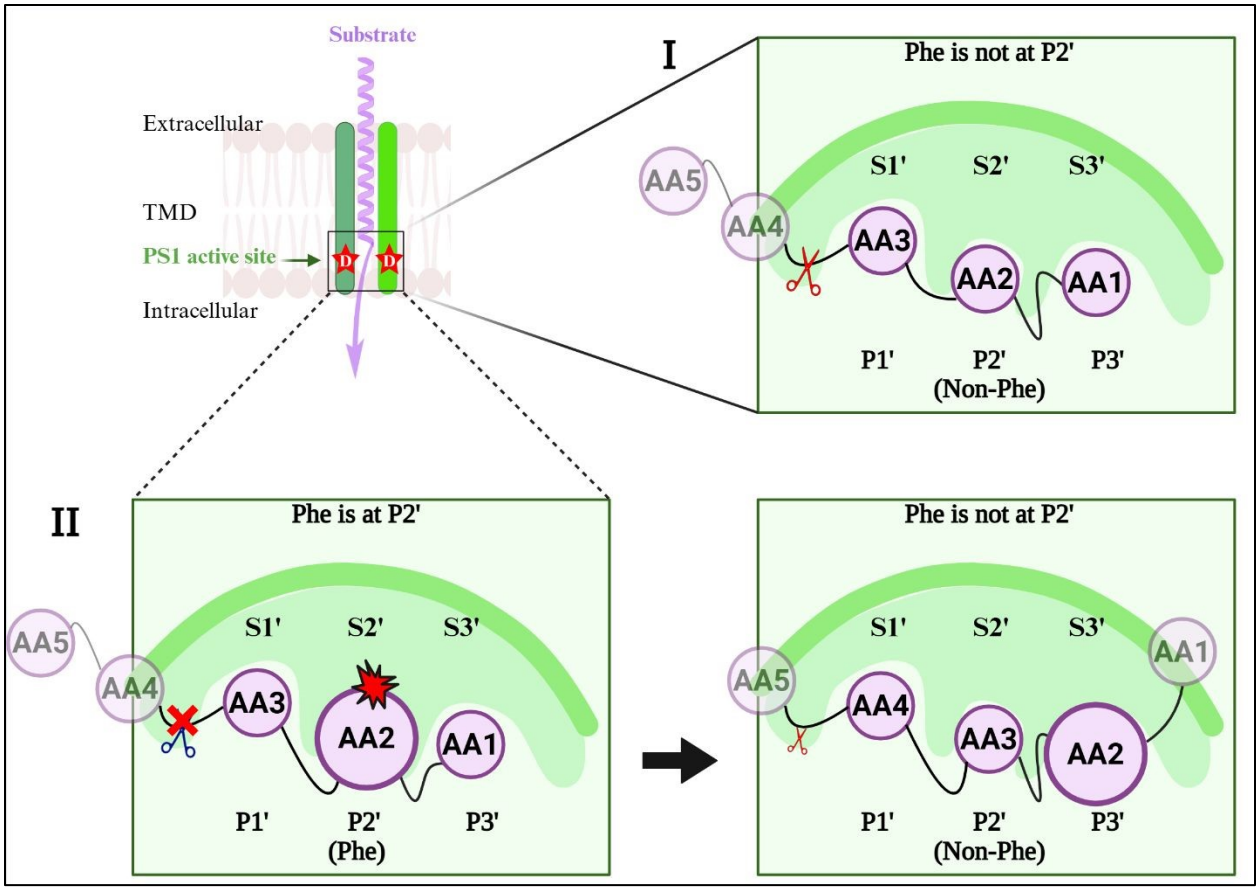

Figure 8: Representation of phenylalanine specificity rule for substrate cleavage by γ-secretase. The active site of PS1 (in green 3 pockets) and two different cleavage scenarios of the substrate (purple) are shown in upper and lower panels. In the upper panel (I), the cleavage by γ-secretase (red scissor) occurs between AA3 and AA4, where AA2 is not Phe and hence Phe is not in P2' position. Three amino acids in the substrate sequence occupy three active site pockets of γ-secretase sequentially. Lower panels (II) show cleavage of the substrate when P2' position is occupied with bulkier Phe (AA2). Bulky Phe in P2' position clashes (red) with small S2' active site pocket. The substrate hence moves further into active site of the enzyme, Phe (AA2) now occupying bigger S3' pocket of active site and therefore gets cleaved between AA4 and AA5 to smaller extent (small red scissor). The scissors represent the peptide bond cleavage site, and AA represents amino acid in the protein sequence. D represents active site aspartates.

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γ- SECRETASE IN ALZHEIMER'S DISEASE

Alzheimer's Disease (AD), is a devastating neurodegenerative disease that causes cognitive decline and is the most common form of dementia.[163] Pathophysiological hallmarks include deposition of cerebral plaques composed of Aβ peptides outside neurons and neurofibrillary tangles composed of filamentous tau protein inside neurons. Aggregation of these misfolded proteins, together with other neuropathologies, is widely thought to cause neuronal degeneration.[164] AD is categorized into two types with varying time of onset: early-onset AD (familial AD, or FAD), and late-onset AD (Sporadic AD, or SAD).[165] FAD accounts for a very small portion of all AD cases.

Multiple factors, including age, genetics, lifestyle, environmental factors, other diseases, and head injuries are linked to increased risk of AD. Therefore, various hypotheses, dependent and independent of Aβ, have been proposed and reviewed in detail.[166,167,168] However, these hypotheses are not mutually exclusive; some could be simultaneously contributing towards the development of AD. All these hypotheses are summarized in Table 1. Based on these various hypotheses, many treatment options for disease management have also been proposed. Despite extensive research, no cure has yet been discovered for the disease. The treatments like acetylcholinesterase inhibitors (AchEIs) or N-methyl D-aspartate (NMDA) receptor antagonists have been used for symptom management and improving quality of life in AD patients. Many clinical trials were done and many are still in progress for AD based on gene therapy[165] and amyloid dependent/ independent hypotheses.[167,168,169,170,171]

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Hypothesis	Explanation
Amyloid cascade Hypothesis[172-175]	Aβ42 aggregates to form extracellular Aβ plaques in the brain
Tau aggregation Hypothesis[202-205]	Aggregation of hyperphosphorylated tau proteins causes neurofibrillary tangles in the brain
Mitochondrial cascade Hypothesis[207-208]	Dysfunctional mitochondria cause increase in reactive oxygen species, in turn causing neuronal damage
Genetic Hypothesis[213-217]	ApoE4 allelic variant as genetic risk factor
Stalled E-S complex Hypothesis[218-219]	FAD-mutant presenilin or APP substrate form a stable γ-secretase enzyme-substrate complex triggering synaptic degeneration
Vascular Hypothesis[223-226]	Dysfunctional cerebrovascular system reduces blood supply to brain
Infection Hypothesis[227-228]	Microbial infection in the brain
Neurotransmitter Hypothesis[166-167]	Imbalance of neurotransmitters such as acetylcholine and glutamate
Metal Homeostasis Hypothesis[166]	Dysregulation of essential and toxic levels of non-essential metal ions

Abbreviations: AD-Alzheimer's Disease; Aβ- Amyloid β; ApoE- apolipoprotein E.

Table 1: The various hypotheses of Alzheimer's Disease (AD)

Amyloid Hypothesis

The amyloid cascade hypothesis, first proposed in 1991, is the most widely accepted explanation of AD pathogenesis.[172,173,174] Its most recent formulation states that soluble Aβ oligomers initiate AD and that other pathological features result from Aβ aggregation.[175] As a corollary, symptoms of AD were predicted to be improved with reduction in Aβ and plaques level,[176] which mainly consists of Aβ42.[177,178] Dominant missense mutations in APP (generally found in and around the small Aβ region of APP) and in PS1 and PS2 were discovered to be associated with FAD, and these mutations were soon found to increase the proportion of aggregation-prone Aβ42.[179,180,181,182] In contrast, SAD appears to involve the failure to clear

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generated Aβ, leading to increased Aβ42 deposition in the brain as evidenced in human study.[175,183,184] In either case, increased cerebral Aβ42 levels leads to oligomerization of Aβ42 and deposition as plaques, with the oligomers implicated in synaptic dysfunction and the plaques thought to serve as a reservoir for oligomers (Fig. 9).[185] Synaptic dysfunction is followed by synaptic loss, neurodegeneration, and ultimately dementia.[175]

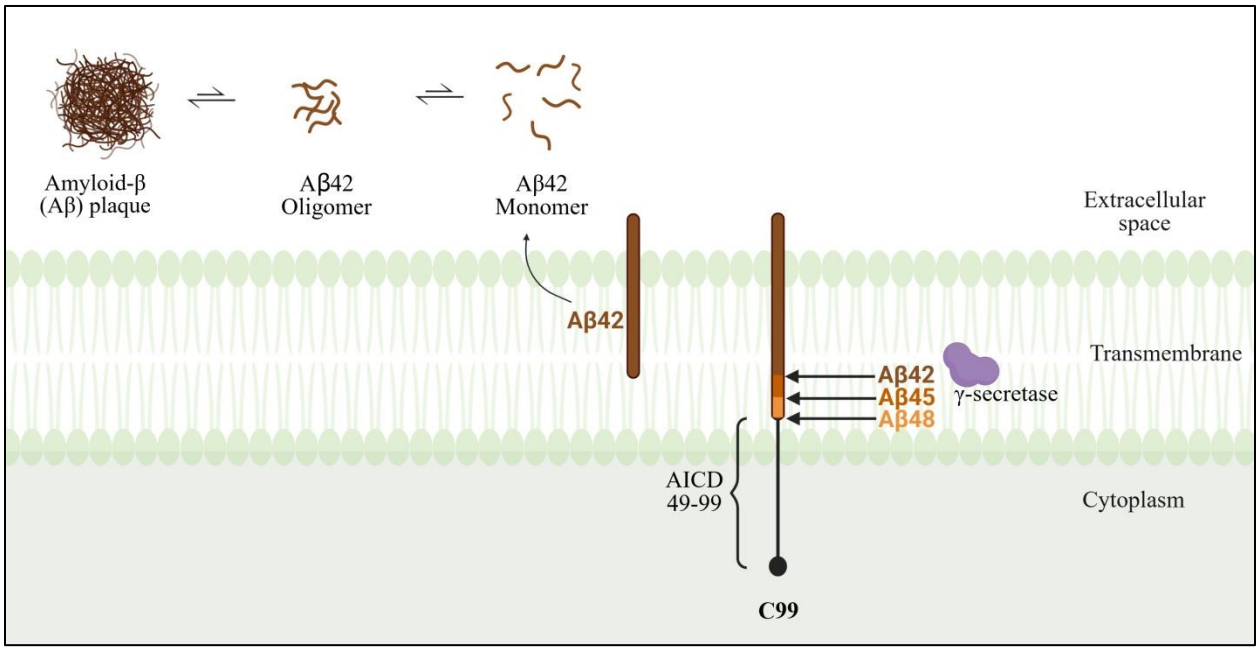

Figure 9: Formation of amyloid β (Aβ) plaques. Generation of Aβ42 from Aβ48 and AICD 49-99 by γ-secretase cleavage is shown. The Aβ42 monomers are released in extracellular space, which clump together to form oligomers which aggregate and deposit in the form of Aβ plaques.

The amyloid cascade hypothesis was further supported by identification of a missense APP mutant (A673T) near the β-secretase cleavage site, which decreases cleavage at this site and provides protection against AD and age-related cognitive decline.[186] Aβ oligomers have been reported to elicit different pathways to impart neuronal toxicity in AD.[166] Based on the amyloid hypothesis, many clinical trials targeting Aβ have been conducted and most (such as BACE and γ-secretase inhibitors) were unsuccessful because of the severe side effects.[187,188] . Another reason for the failure of anti-amyloid trials is that the treatments are not provided early during the disease

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progression. The clinical study in dominantly inherited AD patients had shown that the AD biomarkers such as reduction of Aβ42 and increase of tau protein in CSF (cerebrospinal fluid) as well as deposition of Aβ in plaques appears decades before onset of AD symptoms.[189] The recent development of passive anti-amyloid immunotherapies, however, had shown to be promising.[190,191] The clinical trials of anti-amyloid monoclonal antibodies (MABs) have shown that MAB treatments cleared plaques and decreased the rate of cognitive decline, albeit modestly, providing further support for amyloid hypothesis and the idea that amyloid is viable target for AD treatment.[192,193] Recently, aducanumab, lecanemab, and donanemab were approved by the FDA[194,195] as disease-modifying therapies (DMTs) for the treatment of early AD and mild cognitive impairment (MCI)[192] and many are currently under development.[196] In addition to developing DMTs for symptomatic AD patients, the new Aβ targeted trials including MABs are also focusing on primary and secondary prevention in asymptomatic and presymptomatic high risk individuals carrying genetic mutations.[197] A recent clinical study of long-term gantenerumab treatment in high-risk dominantly inherited AD (DIAD) individuals have shown potential in delaying onset of AD symptoms.[198] However, recent *in vitro* studies on effects of FAD mutations in APP and PS1 revealed that not all mutations increase the Aβ42/Aβ40 ratio.[199,200] Although Aβ appears to be centrally involved in AD, therapies that target only Aβ may be insufficient.[201]

Amyloid-independent hypotheses

Despite huge research efforts focused on the amyloid hypothesis, the failure to find effective Aβ-targeting therapeutics have led to consideration of other hypotheses and formulation of alternative therapeutic strategies. In contrast to the amyloid cascade hypothesis, many of these alternative hypotheses posit that amyloid plaque deposition in the AD brain is a consequence, not the cause, of other pathological changes.

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The tau aggregation hypothesis of AD is perhaps the second-most accepted, as deposition of tau-containing neurofibrillary tangles (NFTs) is one of the hallmark pathological features of AD. This hypothesis is based on accumulation of NFTs generated from aggregation of hyperphosphorylated tau proteins. Tau proteins have different isoforms resulting from alternative splicing and are essential components that provide microtubule stability and integrity.[202,203] Although different pathologies, amyloid and tau aggregation may work together towards AD pathogenesis and progression.[166] Aberrant phosphatase and kinase activity leads to tau hyperphosphorylation, which in turn aggregates and deposits as NFT.[202,204] Abnormal tau truncation, through proteolysis, is another cause of tau aggregation.[205] Additionally, other tau modifications, such as acetylation, nitration, and glycosylation could also contribute to tau aggregation.[205] Furthermore, pathological tau aggregates can apparently spread from neuron to neuron via a seeding mechanism, leading to spread throughout the brain and associated neuronal loss.[203]

Mitochondria act as a 'powerhouse' of the cell, supplying energy in the form of adenosine triphosphate (ATP) through oxidative phosphorylation. Efficient functioning of mitochondria is particularly essential in neurons, as they have high energy requirements.[206] The mitochondrial cascade hypothesis, first proposed in 2004,[207] suggests that mitochondrial dysfunction and resulting increase in reactive oxygen species (ROS) causes neuronal damage, hence contributing to AD pathogenesis. Mitochondrial dysfunction—involving dysregulation of mitochondrial fusion and fission, trafficking, and mitophagy—is observed in AD brain.[206] Mitochondrial dysfunction is reported to affect APP expression, processing and amyloid deposition, in addition to affecting tau phosphorylation, inflammation and oxidative stress.[208] Oxidative stress occurs from disrupted redox systems, where imbalance between biological oxidants and antioxidants is observed[209] and

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involves increased production of ROS or other reactive species which causes deterioration of neuronal cells.[210] In AD, the activity of mitochondrial enzymes in the oxidative pathway is altered,[211] and the number of mitochondria is reduced. Moreover, abnormal mitochondrial DNA is associated with AD.[212]

Besides the FAD mutations found in APP and PS, allelic variation of apolipoprotein E (ApoE), is a major genetic risk factor for AD. The ApoE protein acts as a lipid carrier and is involved in lipid metabolism.[213] Of the three ApoE alleles found in humans, ε2 (ApoE2), ε3 (ApoE3) and ε4 (ApoE4), with differences only at amino acids 112 and 158, ε4 is strongly associated with AD.[213,214,215] ApoE4 is associated with reduced Aβ clearance in the brain,[215] increased hyperphosphorylation of tau.[216] and stabilization of Aβ oligomers.[214] Additionally, variants such as R47H in the microglial transmembrane protein TREM2 are reported to be another genetic risk factor contributing to AD development.[217]

Recently the "stalled enzyme-substrate (E-S) complex" hypothesis was proposed for FAD, which posits that FAD mutations in either presenilin (the catalytic component of γ-secretase) or APP (one of its substrates) may lead to stabilized E-S complexes that trigger synaptic degeneration. These stalled E-S complexes lead to deficient processing of substrates, producing increased proportions of long Aβ peptides in the case of APP C99 substrate, thereby generally increasing the Aβ42/Aβ40 ratio. A *C. elegans* model system for FAD was developed and leveraged to show that FAD mutations trigger synaptic degeneration either through deficient processing of other essential substrates (i.e., loss of function) or through the stalled E-S complex *per se* (i.e. gain of toxic function).[218,219] However, a dominant loss-of-function mechanism alone can be ruled out, as PS1 mutants in the human population that lead to nonsense-mediated decay of the mRNA (and therefore haploinsufficiency) cause a hereditary skin disease, not neurodegeneration.[220] Moreover,

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in the *C. elegans* model system, transgenic lines expressing catalytically dead PS1 (D257A) do not display a neurodegenerative phenotype as seen with FAD mutations.[221] The *C. elegans* system further revealed that APP C99 mutations that block Aβ production and comparable Notch "N99" mutations that reduce ε cleavage likewise trigger synaptic degeneration, suggesting that stalled substrates other than APP can be neurotoxic. Consistent with these findings, a mouse knock-in model with an FAD-mutant PS1 (L435F) was recently found to develop age-dependent neurodegeneration even when the *APP* gene was knocked out.[222]

The vascular hypothesis suggests that the dysfunctional cerebrovascular system may synergistically contribute to development of AD.[223] Clinical studies suggests that cerebral vascular dysfunction and reduced blood flow occurs before appearance of hallmarks of AD.[224, 225] Vascular diseases like hypertension, diabetes, hyperlipidemia, and hypercholesterolemia have been linked with the occurrence and progression of AD in mouse and clinical studies.[226] These diseases cause damage in the cerebral vasculature, which leads to brain dysfunction and neurodegeneration in AD.

Another hypothesis connects microbes, microbial infection and neuroinflammation to sporadic AD pathogenesis.[227,228] Considerable evidence suggest that Aβ peptides have antimicrobial activity[229,230, 231] and their synthesis is increased in microbial infections.[227] Age-dependent altered gut microbiota is also connected to dementia[232] as well as to increased permeability and dysfunction of the blood brain barrier (BBB),[233] and hence with onset of AD.

Some older hypotheses propose association of an imbalance of neurotransmitters, such as acetylcholine or glutamate, as well as calcium homeostasis with the development of AD.[166,167] The cholinergic hypothesis links decreased synaptic acetylcholine—a neurotransmitter involved in memory and cognitive functions—with AD, while the glutamatergic hypothesis links overactivity

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of ionotropic NMDA-type glutamine receptors to AD. In addition to calcium, dysregulation of other essential metal ions such as zinc, iron, and copper as well as toxic intake of non-essential metal ions such as aluminum, lead, and cadmium are reported to be associated with AD pathology.[166] Other than these, amyloid cross-seeding, lymphatic system, microRNA, ion channel, cell cycle, autoimmune, granuloma, dysregulated Reelin homeostasis, pesticide-induced neuropathology hypotheses have also been proposed.[166,167,234,235,236] Many recent reviews propose integration of multiple hypothesis and their concurrent and interdependent occurrence.[237,238,239]

THE ROLE OF γ-SECRETASE IN BIOLOGY AND DISEASES OTHER THAN AD

Normal γ-secretase functioning is essential during embryonic development as well as adult well-being. As mentioned, γ-secretase has many type I integral membrane substrates besides APP. Given the wide range of substrates, the enzyme is involved in a broad range of biological functions (Fig. 10).[68,240] γ-Secretase-mediated pathways are associated with various biological systems (e.g., nervous, cardiovascular, skin, kidney, and immune), and hence γ-secretase is involved in many diseases related to these systems as well as cancers (Fig. 10).[240] The functions of γ-secretase generally can be categorized into degradation and clearing of the membrane proteins, extracellular release of shorter peptides from substrates, cell signaling by released ICDs, or termination of action of full-length substrates, depending upon the substrate cleaved (Fig. 10). For example, it was

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suggested recently that γ-secretase not only generates physiologically active fragments from substrates but also inactivates neurotoxic C99.[118]

The membrane-associated C-terminal stubs that remain after ectodomain shedding require clearance from the membrane, and γ-secretase is proposed to perform this critical degradation function, hence acting as the 'proteasome of the membrane'.[107] This cleavage generates secreted N-terminal peptides as well as C-terminal intracellular domains (ICDs), which may themselves serve some biological roles. The AICD, generated after ε-cleavage of APP by γ-secretase, has

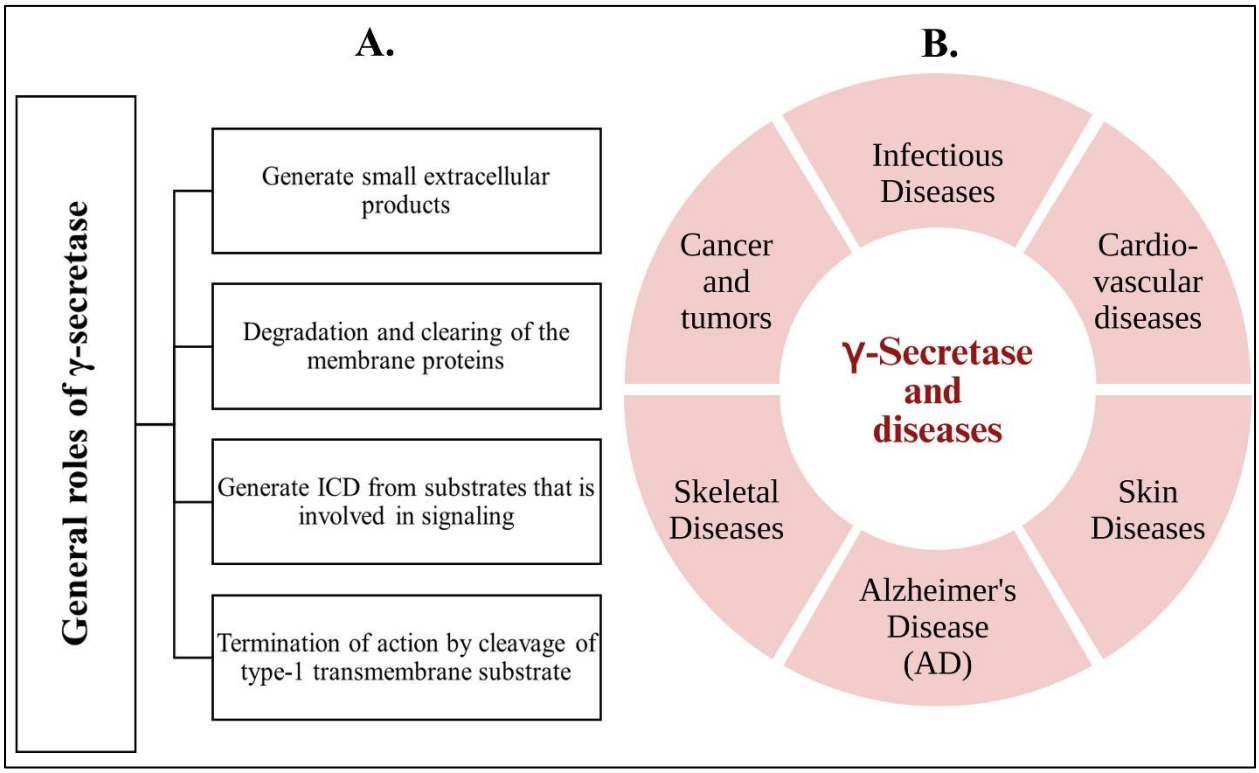

Figure 10: The role of γ-secretase in biology and diseases other than AD

been proposed to have many roles, including transcription regulation,[241,242] but these remains controversial. The NICD generated from Notch family receptor cleavage is involved in gene transcription, as noted earlier. Besides Notch, ICDs from alcadeins, cadherins, Notch ligands Delta and Jagged, CD44 and many other substrates have also been reported to translocate to the nucleus

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and affect transcription.[68,101] Furthermore, alcadein-α ICD is involved in regulating the trafficking of membrane proteins, specifically APP.[243]

γ-Secretase is also known to be critically involved in regulating inflammatory responses and innate immunity.[244] For example, γ-secretase is involved in an adipocyte-mediated inflammatory signaling pathway and regulation of adipose tissue inflammation, likely via IL-6.[245] γ-Secretase also cleaves cell-adhesion proteins to regulate the assembly or disassembly of adhesion junctions. For example, cleavage of E-cadherin by γ-secretase promotes dissociation of adherens junctions.[136] γ-Secretase is found in pre- as well as post-synapses, where it is involved in synaptic transmission and autoregulation[246] as well as helping to maintain synaptic plasticity.[247] Altered γ-secretase processing of neuregulin-1 (NRG-1) is strongly linked with an increased risk of schizophrenia.[248]

Dysregulated Notch signaling is implicated in cancers of various biological systems including nervous, digestive, respiratory, blood, reproductive, and urinary systems.[124] The CD-44-ICD was shown to possess oncogenic cell transformation activity affecting tumorigenesis.[249] Protein-tyrosine kinase 7 (PTK7) is a regulator of planar cell polarity and upregulated in many cancers. Importantly, the ICD generated from its γ-secretase cleavage translocates to the nucleus and enhances tumorigenesis.[250] The process of angiogenesis is physiologically essential for development and tissue repair but pathological angiogenesis is involved in diseases like cancer. γ-Secretase has been reported to play a key role in regulating angiogenesis through various factors such as Notch, ErbB4, and insulin-like growth factor-1 receptor (IGF1-R), vascular endothelial growth factor receptor-1 (VEGFR-1), cadherin, and APP.[251]

γ-Secretase is also involved in cardiac development as well as maintenance and regulation of cardiac functions via many of its substrates such as Notch1, the NRG1-ErbB4 pathway, subunits of voltage-gated sodium and potassium channels.[252] Normal γ-secretase functioning is essential

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for maintaining cardiac health, as various cardiac disorders were observed in γ-secretase knockout animal models.[252] γ-Secretase also plays a role in metabolism and metabolic diseases via Notch signaling.[253] Interestingly, hepatic γ-secretase regulates cleavage of the low-density lipoprotein receptor (LDLR) and affects VLDL/LDL uptake in a Notch-independent manner.[254] Normal Notch signaling, and hence γ-secretase activity, is physiologically essential in bone biology, and both Notch overexpression as well as loss of Notch activity are involved in bone pathology in mice.[255] Notch plays a critical role during skeletal development by regulating the differentiation and function of osteoblast and osteoclast cells.[256,257]

γ-Secretase also plays a vital role in skin biology, and its inhibition can cause skin abnormalities.[220,258] γ-Secretase likely acts through various pathways in skin biology, including Notch, EGFR and phosphoinositide-3-kinase (PI3K).[259] About 57 genetic mutations in γ-secretase components PS1, NCT, and PEN-2 have been reported to date that cause a debilitating inflammatory skin disease called acne inversa (AI) or hidradenitis suppurativa (HS).[260]

γ-Secretase is also proposed to be involved in infectious diseases. The proteolytic activity of γ-secretase was reported to be required for efficient human cytomegalovirus (HCMV) replication at the transcriptional level, independent of Notch signaling.[261] Interestingly, for human papillomavirus (HPV) infections, although γ-secretase is necessary, its proteolytic action is not.[262] In HPV infections, γ-secretase serves a chaperone function and promotes insertion of L2 capsid protein in endosomal membranes at low pH.[262]

In addition to their role in the γ-secretase complex and enzymatic activity, the individual γ-secretase components are reported to have other non-proteolytic roles in biology.[263] For instance, PS can act as a scaffolding protein in mammals as well as plants and amoeba.[264] PS is associated with other roles as well, such as calcium homeostasis, autophagy, and protein trafficking/

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degradation, apoptosis, inflammation, and synaptic functions.[263,265] PS mutations are also reportedly involved in neurodegenerative diseases other than AD, such as frontotemporal dementia.[266] APH1 and PEN2 possess protective anti-apoptotic activity via the p53 pathway that is independent of γ-secretase activity.[267] NCT was also found to be cell-protective by controlling cell-death via PI3K/Akt- and p53-dependent pathways, independently of γ-secretase activity.[268]

γ-SECRETASE AS A THERAPEUTIC TARGET

γ-Secretase, first discovered in the context of AD, has been a major therapeutic target for AD for three decades. As Aβ plaques are a pathological hallmark of AD and FAD mutations are found in APP and PS, development of γ-secretase inhibitors (GSI) was of keen interest. Inhibition of γ-secretase activity with a GSI to reduce Aβ production was first demonstrated *in vivo* with dipeptide analog DAPT.[269] Many other GSIs have been developed since then. Sulfonamides (e.g. BMS299897),[270] benzodiazepines (LY-411575)[271] and benzolactams (LY-450139)[272] were developed subsequently that showed reduction in brain Aβ production. However, GSIs also showed concerning toxicities upon chronic treatment, similar to phenotypes observed upon knockout of *Notch1* or *PS1*, suggesting that GSI toxicities are due to inhibition of Notch cleavage and signaling.[271]

In late-stage clinical trials of GSIs semagacestat and avagacestat, adverse effects such as skin cancer and immunosuppression were observed.[273,274] In addition to blocking cleavage of APP, these GSIs also block γ-secretase-mediated Notch cleavage,[275] which likely is the reason for these adverse reactions. Therefore, development of GSIs with selectivity towards APP vs Notch was thought to be a necessity for their therapeutic usefulness as AD treatments. More concerning, however, treatment with GSIs led to cognitive worsening and AD progression.[273,276] Furthermore,

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the clinical failure of GSIs due to worsening of cognitive symptoms correlates with impaired clearance of C99. The resultant increased C99 accumulation may lead to aggravation of disease conditions suggesting, therefore, γ-secretase inhibition is not a good treatment strategy for AD.[118]

Despite their clinical failure as treatments for AD, GSIs are being repurposed to treat many other diseases. γ-Secretase is proposed to be a good therapeutic target for treating excessive angiogenesis; however, development of molecules specific to the substrates involved in this process is needed.[251] Liver-specific GSIs might be effective for treatment of various metabolic diseases characterized by hypertriglyceridemia.[254] GSIs reduce LDLR cleavage, hence stabilizing it and reducing levels of triglyceride-rich lipoproteins in the plasma.[254]

Inhibition of Notch signaling with GSIs has also been reported to improve glucose tolerance and insulin sensitivity, while reducing hepatic glucose production. Hence GSIs may be a beneficial treatment option for diabetes.[277] Recently, liver-targeted GSI-nanoparticle treatment was shown to provide localized hepatic Notch-signaling inhibition, which led to reduced intestinal side-effects with improved obesity-induced glucose tolerance and reduced liver fibrosis in mice.[278] Controlling Notch signaling by downregulation using GSIs could be a useful strategy in certain bone diseases;[255] however, it comes with risks of off-target effects.[256] Furthermore, transient treatment with the dipeptide analog GSI DAPT was shown to enhance bone formation and fracture repair via Notch pathway inhibition in mice, suggesting therapeutic use of GSI in treating skeletal fractures.[279]

Given that certain γ-secretase substrates, especially Notch, are involved in tumorigenesis, many GSIs have also been tested as anti-cancer agents with promising pre-clinical trials, but without much clinical success.[280,281] Recently, the GSI nirogacestat was shown to be beneficial for desmoid tumors in clinical trials[282] and has been approved by FDA as a first targeted therapy for

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this condition. GSI treatment have been shown to improve efficacy of BCMA-targeted CAR-T (chimeric antigen receptor-T) cell therapy in multiple myeloma.[283] GSIs were also shown to inhibit HCMV viral replication and hence are potential anti-HCMV agents.[261]

γ-Secretase modulators (GSMs), on the other hand, could be a safer option to target γ-secretase in AD.[284,] GSMs do not bind in the active site, but instead bind to an allosteric site on the enzyme to modulate γ-secretase activity to lower Aβ42 levels without affecting total Aβ levels (i.e., they are not inhibitors). Specifically, GSMs reduce pathological Aβ42 levels by increasing conversion of Aβ42 to Aβ38.[286,287,288] Importantly, GSMs do not affect proteolysis and signaling of Notch[284] or other substrates.[289] The first GSMs identified were a subset of non-steroidal anti-inflammatory drugs (NSAIDs).

These first-generation GSMs had low potency. Second-generation GSMs such as NSAID-based aryl acetic acids, non-NSAID heterocycles, and natural product analogs apparently have different mechanisms of action and Aβ-altering profiles.[285,290] The heterocyclic GSMs includes arylimidazoles (e.g. E2012, E2212), oxadiazolines and oxadiazines, pyridines, pyrimidines, morpholines and many more.[285] A lead compound from newly discovered triazolo-azepine class of GSMs has shown good pharmacokinetic, toxicological and pharmacodynamic properties and has potential for further advancements.[291] A recent report mentions development of a new pyridazine-based GSM with promising preclinical data and potential for early clinical trials.[292] Another recent report identified GSMs that could be effective not only for sporadic AD but also for FAD, including the aggressive PS1 L166P mutant, which has been resistant to most GSMs.[293]

Although GSMs have potential for development as anti-AD treatment, the efficacy of the GSMs or any other molecules targeting Aβ42 in slowing disease progression in human trials still needs to be demonstrated.[148] Targeting Aβ or γ-secretase alone may not be sufficient for treating

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AD, but combination therapies targeting multiple pathologies and factors at once might be effective.[294]

CONCLUSION

Since its discovery, γ-secretase has been of constant interest to the scientific community. It is the most complicated and promiscuous intramembrane proteolytic enzyme, known for its wide range of substrates. Hence, the enzyme plays essential roles in biology and is involved in myriads of biological functions. Given its importance in human disease, the enzyme has also been a target for development of therapeutics for many pathological conditions for decades, and many are currently in clinical trials. Our understanding since the first description of γ-secretase activity has improved dramatically with biochemical, synthetic and structural approaches. Nevertheless, many uncovered aspects remain to be revealed through extensive research for a full understanding of the enzyme and its use as a successful clinical target.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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