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γ secretase- Molecular Scissors in Alzheimer's Disease

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A B S T R A C T

Gamma secretase is an aspartyl protease enzyme that cleaves the beta-amyloid precursor protein in the beta carboxy-terminal region. In Alzheimer's disease pathogenesis, gamma-secretase is a crucial molecule created by amyloid-beta protein. Catalytic components of proteolytic gamma-secretase complexes are the presenilins that generate amyloid-beta proteins and cell signalling peptides. Substrates of gamma-secretase complexes are mostly membrane-bound peptides developed from outer membrane domains of type I transmembrane proteins thesis has emerged as a phenomenon of exceptional interest in studying gamma-secretase cleavage patterns and enzymatic activity in just the past few years. An understanding of the gamma-secretase role may prove to be essential for AD therapeutics. This study is set out to explore the successive cleavage of gamma-secretase products and their roles in Alzheimer's disease.

Keywords:Alzheimer's disease, gamma-secretase, presenilin, Transmembrane proteins, amyloid precursor protein

Introduction

Proteolytic complex gamma-secretase contains presenilin from its catalytic centre onsite for processing amyloid precursor protein, amyloid plaques and cerebrovascular amyloidosis (CVA). Gamma secretase complexes are mostly transmembrane proteins which are the members of the Disintegrin and metalloproteinase family (Barthet et al., 2012). In Alzheimer's disease pathogenesis, amyloid beta-protein involves distinct molecular pathways named: amyloidogenic. The beta-secretase does extracellular cleavage of the amyloid precursor protein, early counter course amyloid-beta results in the synthesis of amyloid precursor protein-membrane peptide (APP-MP) (Vassar & Kandalepas, 2011). This phenomenon has been widely observed in that gamma-secretase cleaves the APP at different gamma sites generating soluble peptides having various C terminal ends. These amyloid-beta peptides are aggregate to develop neurodegenerative disorders like Alzheimer's and down syndrome (Barthet et al., 2012). In the nonamyloidogenic path, alpha-secretase cleaves the outer APP with the help of metalloproteins in between the amyloid-beta sequence (Ab), which inhibits the amyloid-beta generation (Buxbaum et al., 1998). Evidence from several cohort studies indicated that receptors and proteins present in the cell surfaces are processed the same as nonamyloidogenic processing of APP. Disintegrin and metalloproteinase enzymes cleave the proteins to get C- terminal fragments (CTF1s) of membrane-bound peptides.
Furthermore, cytoplasmic interface proteins are cleaved at epsilon sites by the gamma-secretase enzyme to generate cytosolic proteins named APP intracellular domain(AICD) or CTF2. APP amyloidogenic pathway also produces AICD(Fig). Cell surface proteins signal transduction and gene expression pathways produce polypeptides with essential cellular functions, cleaved by gamma-secretase(Robakis, 2003).
Convincing data from randomized trials have demonstrated that cleavage patterns of gamma-secretase and their molecular mechanisms are coming up now, revealing that various cellular events regulate substrate selection and actions. Researchers' current efforts are to reduce this amyloid beta-protein aggregation by inhibiting the gamma-secretase activity through pharmacological agents, for instance, gamma-secretase inhibitors (GSI). The mechanism of GSI inhibitor is cleaving at epsilon sites of the membrane-bound proteins such as cadherins and Notch1 by inhibiting the functional CTF2 peptides. These consequences result in toxic substance accumulation(Barthet et al., 2012). This paper explores the mechanisms, substrate regulation, products of gamma-secretase, and the molecular mechanisms of amyloid-beta protein development.

Structure of gamma-secretase

In the hydrophobic environment, gamma-secretase cleaves the peptide bonds very efficiently. Nowadays, such cleavages are well studied proteolytic mechanisms within the lipid bilayer with components from serine (rhomboids), aspartic (PSEN), and zinc metal protease (S2- protease)(Beel & Sanders, 2008). Furthermore, our understandings are very limited to the actions of gamma-secretase on intramembrane proteins. Gamma secretase includes four hydrophobic proteins and 19 transmembrane domains (TMD) with challenging structural and functional characterization(De Strooper, 2003). In Alzheimer's disease pathogenesis, gamma-secretase plays a crucial role in the production of amyloid beta-protein by amyloid precursor protein processing. Hence, gamma-secretase modulation is needed to reduce this amyloid-beta protein and provide a high pharmacological therapeutic index(Selkoe & Wolfe, 2007).

Subunits of gamma-secretase

Presenilin

Presenilin genes (PSEN 1 and PSEN 2) and their proteins (PS1 and PS2) were identified through genetic linkage analysis related to familial AD (FAD). Notch phenotype was identified in Pen 1 knockout mice(Szmeja, 2008). The above results are helped to conclude PSEN1 gene was essential for gamma-secretase activity by reduced production of amyloid beta-protein and accumulation of C- terminal fragments of APP(De Strooper, 2003). PSEN2 is continuous with the balance of molecular processes(Herreman et al., 2000). In recent studies, two conserved aspartates were identified in PSEN, responsible for proteolysis, recommended that it is an aspartic protease that cleaves the APP directly(Selkoe & Wolfe, 2007)(Wolfe et al., 1999). Whereas the activity of PSEN depends on various limiting factors present inside the cell(Annaert & De Strooper, 2002) that led to the recognition of three or more needed adequate proteins(Edbauer et al., 2003) essential for gamma-secretase activity; Nicastrin, PEN- 2 and APH- 1.

Nicastrin

Nicastrin was shown to be a significant PSEN interacting protein identified biochemically by affinity chromatography(Yu et al., 2000). With the help of its Transmembrane, the primary interaction is made between PSEN and APH- 1 because Nicastrin is a Type I glycoprotein(Morais et al., 2003). Various N- glycosylation sites are present in the large ectodomain region; these glycosylation sites interfere with enzyme activity(Herreman et al., 2003). The incorporation of gamma-secretase made conformational changes in the ectodomain regions and had a DYGIS motif. It is present in the more significant part of the ectodomain, named the DAP domain, which exhibits structural similarities with the aminopeptidases; on the other hand, nicastrin doesn't indicate any aminopeptidase action(Fagan et al., 2001).

Gamma secretase possesses an initial substrate binding site as the DAP region; in those regions, specific interaction is made between the substrates amino terminus with glutamic residue (Glu 333) of gamma-secretase(Shah et al., 2005). Current studies revealed that this residue is a disturbance for gamma-secretase maturation rather than substrate identification. Gamma secretase with Glu- 333 inactivated other regions of the enzyme but on a molecular essential remaining domain were active in different assays; this statement reopened the debate about Nicastrin's particular position in the secretase complex(Chávez-Gutiérrez et al., 2008).

APH- 1 and PEN- 2

APH-1 and PEN- 2 encoding genes were identified in *C. elegans* during PSEN interactors genetic screening(Goutte et al., 2002). There are three different APH genes in rodents(Aph1a, Aph1b, and Aph1c) and two in humans (APH1a and APH1b), the duplication process in rodents makes another copy of Aph1c, which has 96% similarity(Chávez-Gutiérrez et al., 2008). The APH-1 proteins have N and C- terminus facing the luminal side of the cytosol with seven Transmembrane domains. TM4 has a conserved Gxxg motif that interacts with the PSEN1 and PSEN2(Niimura et al., 2005). The above proteins play a crucial role as a stable component during protein assembly, but the part in the gamma-secretase activity is still unclear.

PEN- 2 has two transmembranes, and it looks like a small hairpin membrane protein. Interaction with PSEN2 has been made with outer cellular N-terminus of the PEN- 2 protein(Fraering, 2008), whereas in endoproteolysis C- terminus of the protein and TM1 interaction is necessary, the parallel activation of PSEN is still unknown.

In the early endoplasmic reticulum, the complex assembly starts with Nicastrin and APH-1 and then is attached with the PSEN and PEN-2 complex. Regulation of this assembly in the ER and held in the ER- Golgi recycling and cargo sorting mechanism. The recovery of Nicastrin and PEN- 2 unassembled proteins were identified in the cis- Golgi network controlled by Rer1p(Spasic et al., 2007). Immature Nicastrins are recycled back to the ER by Rer1p binding. Further interactions hide the binding site of Rer1p, which allows the exit way of Nicastrin- APH1 complexes and then associates with the PS and PEN- 2 complex, organizing the active gamma-secretase(Spasic et al., 2007).

Revolutions in cryo-EM technology made it easy to visualize complex structures like gamma-secretase(Quentin & Raunser, 2018). Developments in cryo-EM led to the study of the detailed design of gamma-secretase complex exhibits horseshoe-shaped arrangement with nineteen Trans Membrane Domains (TMD). The subunit Nicastrin location was identified(Lu et al., 2014). Continuous studies reveal that horseshoe-shaped structure of convex side the active

site residues are present(Bai, Yan, et al., 2015). The Nicastrin ectodomain has operational sites and prevents the substrate-binding with extracellular domains. New biochemical studies show that shorter extracellular domains were processed faster than the high-affinity binding of substrate TMD by gamma-secretase because of the nicastrin. Nicastrin processed longer substrates by reducing disulfide bonds(Bolduc et al., 2016). TMD of PSEN2 has been found not to go through the membrane. PSEN 2 directly interacted with TMD 4 of PSEN 1 with consistent mutagenesis studies.

Enzymatic complexes of gamma-secretase

It should be noted that previous studies have indicated that the structure of gamma-secretase has flexible complexes, and this flexibility is reduced at the time of gamma-secretase inhibitor binding. These results were analyzed under the low-resolution electron microscope(Li et al., 2014). Mass spectrometric research revealed that single-pass transmembrane proteins could be co-purified with gamma-secretase (Bai, Rajendra, et al., 2015). More recent work by (Zhou et al., 2019) showed the enzyme-substrate interaction of gamma-secretase by APP-derived substrate C83 with exciting insights. This stable substrate complex was achieved by the formation of a disulfide bridge between C83 and the hydrophilic loop of Presenilin1 (PS1) (Zhou et al., 2019). the β-secretase activity was removed by modifying the catalytic aspartate D385 into alanine to avoid substrate cleavage. This enzyme-substrate Complex was analyzed under a cryo-electron microscope with a 2.6 A resolution that resembles just before the photolytic process(Zhou et al., 2019).

Conclusion

For treating Alzheimer's disease, gamma-secretase is a potential drug target and attracts research interests. Current research focuses on the γ secretase modulators and inhibitors to inhibit the Amyloid Precursor Protein Cleavage. To identify the enzyme's active site, substrate-binding and allosteric binding site modulators and inhibitors play a crucial role. Present drugs and therapies are not sufficient for Alzheimer's disease. Several new medicines and treatments are needed to reduce AD pathology with reduced side effects.

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References

Annaert, W., & De Strooper, B. (2002). A cell biological perspective on Alzheimer's disease. *Annual Review of Cell and Developmental Biology*, *18*, 25–51. https://doi.org/10.1146/annurev.cellbio.18.020402.142302

Bai, X. C., Rajendra, E., Yang, G., Shi, Y., & Scheres, S. H. W. (2015). Sampling the conformational space of the catalytic subunit of human g-secretase. *ELife*, *4*(December2015), 1–19. https://doi.org/10.7554/eLife.11182

Bai, X. C., Yan, C., Yang, G., Lu, P., Ma, D., Sun, L., Zhou, R., Scheres, S. H. W., & Shi, Y. (2015). Anatomic structure of human γ-secretase. *Nature*, *525*(7568), 212–217. https://doi.org/10.1038/nature14892

Barthet, G., Georgakopoulos, A., & Robakis, N. K. (2012). Cellular mechanisms of γ-secretase substrate selection, processing and toxicity. *Progress in Neurobiology*, *98*(2), 166–175. https://doi.org/10.1016/j.pneurobio.2012.05.006

Beel, A. J., & Sanders, C. R. (2008). Substrate specificity of γ-secretase and other intramembrane proteases. *Cellular and Molecular Life Sciences*, *65*(9), 1311–1334. https://doi.org/10.1007/s00018-008-7462-2

Bolduc, D. M., Montagna, D. R., Gu, Y., Selkoe, D. J., & Wolfe, M. S. (2016). Nicastrin functions to sterically hinder γ-secretase-substrate interactions driven by the substrate transmembrane domain. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(5), E509–E518. https://doi.org/10.1073/pnas.1512952113

Buxbaum, J. D., Liu, K. N., Luo, Y., Slack, J. L., Stocking, K. L., Peschon, J. J., Johnson, R. S., Castner, B. J., Cerretti, D. P., & Black, R. A. (1998). Evidence that tumour necrosis factor α converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *Journal of Biological Chemistry*, *273*(43), 27765–27767. https://doi.org/10.1074/jbc.273.43.27765

Chávez-Gutiérrez, L., Tolia, A., Maes, E., Li, T., Wong, P. C., & De Strooper, B. (2008). Glu332 in the nicastrin ectodomain is essential for γ-secretase complex maturation but not for its activity. *Journal of Biological Chemistry*, *283*(29), 20096–20105. https://doi.org/10.1074/jbc.M803040200

De Strooper, B. (2003). Aph-1, Pen-2, and Nicastrin with Presenilin generate an active γ-Secretase complex. *Neuron*, *38*(1), 9–12. https://doi.org/10.1016/S0896-6273(03)00205-8

Edbauer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., & Haass, C. (2003). Reconstitution of γ-secretase activity. *Nature Cell Biology*, *5*(5), 486–488. https://doi.org/10.1038/ncb960

Fagan, R., Swindells, M., Overington, J., & Weir, M. (2001). Nicastrin, a presenilin-interacting protein, contains an aminopeptidase/transferrin receptor superfamily domain. *Trends in Biochemical Sciences*, *26*(4), 213–214. https://doi.org/10.1016/S0968-0004(01)01789-3

Fraering, P. (2008). Structural and Functional Determinants of γ-Secretase, an Intramembrane Protease Implicated in Alzheimers Disease. *Current Genomics*, *8*(8), 531–549. https://doi.org/10.2174/138920207783769521

Goutte, C., Tsunozaki, M., Hale, V. A., & Priess, J. R. (2002). APH-1 is a multipass membrane protein essential for the Notch signaling pathway in Caenorhabditis elegans embryos. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(2), 775–779. https://doi.org/10.1073/pnas.022523499

Herreman, A., Serneels, L., Annaert, W., Collen, D., Schoonjans, L., & De Strooper, B. (2000). Total inactivation of γ-secretase activity in presenilin-deficient embryonic stem cells. *Nature Cell Biology*, *2*(7), 461–462. https://doi.org/10.1038/35017105

Herreman, A., Van Gassen, G., Bentahir, M., Nyabi, O., Craessaerts, K., Mueller, U., Annaert, W., & De Strooper, B. (2003). γ-Secretase activity requires the presenilin-dependent trafficking of nicastrin through the Golgi apparatus but not its complex glycosylation. *Journal of Cell Science*, *116*(6), 1127–1136. https://doi.org/10.1242/jcs.00292

Li, Y., Lu, S. H. J., Tsai, C. J., Bohm, C., Qamar, S., Dodd, R. B., Meadows, W., Jeon, A., McLeod, A., Chen, F., Arimon, M., Berezovska, O., Hyman, B. T., Tomita, T., Iwatsubo, T., Johnson, C. M., Farrer, L. A., Schmitt-Ulms, G., Fraser, P. E., & St George-Hyslop, P. H. (2014). Structural interactions between inhibitor and substrate docking sites give insight into mechanisms of human PS1 complexes. *Structure*, *22*(1), 125–135. https://doi.org/10.1016/j.str.2013.09.018

Lu, P., Bai, X. C., Ma, D., Xie, T., Yan, C., Sun, L., Yang, G., Zhao, Y., Zhou, R., Scheres, S. H. W., & Shi, Y. (2014). Three-dimensional structure of human γ-secretase. *Nature*, *512*(7513), 166–170. https://doi.org/10.1038/nature13567

Morais, V. A., Crystal, A. S., Pijak, D. S., Carlin, D., Costa, J., Lee, V. M. Y., & Doms, R. W. (2003). The Transmembrane Domain Region of Nicastrin Mediates Direct Interactions with APH-1 and the γ-Secretase Complex. *Journal of Biological Chemistry*, *278*(44), 43284–43291. https://doi.org/10.1074/jbc.M305685200

Niimura, M., Isoo, N., Takasugi, N., Tsuruoka, M., Ui-Tei, K., Saigo, K., Morohashi, Y., Tomita, T., & Iwatsubo, T. (2005). Aph-1 contributes to the stabilization and trafficking of the γ-secretase Complex through mechanisms involving intermolecular and intramolecular interactions. *Journal of Biological Chemistry*, *280*(13), 12967–12975. https://doi.org/10.1074/jbc.M409829200

Quentin, D., & Raunser, S. (2018). Electron cryomicroscopy as a powerful tool in biomedical research. *Journal of Molecular Medicine*, *96*(6), 483–493. https://doi.org/10.1007/s00109-018-1640-y

Robakis, N. K. (2003). An Alzheimer's disease hypothesis based on transcriptional dysregulation. *Amyloid*, *10*(2), 80–85. https://doi.org/10.3109/13506120309041729

Selkoe, D. J., & Wolfe, M. S. (2007). Presenilin: Running with Scissors in the Membrane. *Cell*, *131*(2), 215–221. https://doi.org/10.1016/j.cell.2007.10.012

Shah, S., Lee, S. F., Tabuchi, K., Hao, Y. H., Yu, C., LaPlant, Q., Ball, H., Dann, C. E., Südhof, T., & Yu, G. (2005). Nicastrin functions as a γ-secretase-substrate receptor. *Cell*, *122*(3), 435–447. https://doi.org/10.1016/j.cell.2005.05.022

Spasic, D., Raemaekers, T., Dillen, K., Declerck, I., Baert, V., Serneels, L., Füllekrug, J., & Annaert, W. (2007). Rer1p competes with APH-1 for binding to nicastrin and regulates γ-secretase complex assembly in the early secretory pathway. *Journal of Cell Biology*, *176*(5), 629–640. https://doi.org/10.1083/jcb.200609180

Szmeja, Z. (2008). Recenzja podręcznika pt. „CT Teaching Manual. A Systematic Approach to CT Reading". *Otolaryngologia Polska*, *62*(1), 117. https://doi.org/10.1016/s0030-6657(08)70226-9

Vassar, R., & Kandalepas, P. C. (2011). The -secretase enzyme BACE1 as a therapeutic target for Alzheimer's disease. *Alzheimer's Research and Therapy*, *3*(3), 1–6. https://doi.org/10.1186/alzrt82

Wolfe, M. S., Selkoe, D. J., Xia, W., Ostaszewski, B. L., Diehl, T. S., & Kimberly, W. T. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and [gamma]-secretase activity : Abstract : Nature. *Nature*, *398*(6727), 513–517. http://www.nature.com/doifinder/10.1038/19077%5Cnpapers3://publication/doi/10.1038/19077

Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A., Song, Y. Q., Rogaeva, E., Chen, F., Kawarai, T., Supala, A., Levesque, L., Yu, H., Yang, D. S., Holmes, E., Milman, P., Liang, Y., Zhang, D. M., Xu, D. H., … George-Hyslop, P. S. (2000). Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and βAPP processing. *Nature*, *407*(6800), 48–54. https://doi.org/10.1038/35024009

Zhou, R., Yang, G., Guo, X., Zhou, Q., Lei, J., & Shi, Y. (2019). Recognition of the amyloid precursor protein by human g-secretase. *Science*, *363*(6428). https://doi.org/10.1126/science.aaw0930