NEURAL REGENERATION RESEARCH
www.nrronline.org

Neurotrophin receptors, gamma-secretase inhibitors, and neurodegeneration of basal forebrain cholinergic neurons

Marçal Vilar*

The amyloid hypothesis of Alzhemer's disease (AD) postulates that the generation of amyloid-beta (Aβ) peptide from the amyloid precursor protein (APP) by the action of the γ-secretase complex is one of the principal causes of AD. This idea is supported by the identification of several hereditary mutations in the *APP* gene or in the *PSEN1* and *PSEN2* genes that encode Presenilin-1 and Presenilin-2, the catalytic component of the γ-secretase complex. The assumption at that time was that familial AD (FAD), mutations lead to a gain of function phenotype, increasing the ratio between the levels of the toxic $A\beta_{1-42}$, and the less toxic $A\beta_{1-40}$ peptide (Kuperstein et al., 2010). Actually, the ratio $A\beta_{1-42}/A\beta_{1-40}$ is the principal cause of the toxic effect as some FAD mutations in APP decrease the levels of $A\beta_{1-40}$ without changing the total levels of $A\beta_{1-42}$ (Ancolio et al., 1997). Pharmaceutical companies started the race to design new highly specific γ-secretase inhibitors (GSIs) with good pharmacological properties to jump into the clinic. The reduction of the circulating levels of $A\beta_{1-42}$ in AD mouse models rapidly supported this approximation, and one of the lead compounds, Semagacestat (Lilly), entered the phase III clinical trials. However, results were not as good as initially expected, and the appearance of several skin cancer problems and a reduction, rather than an improvement, in the cognitive performance of the patients lead the FDA to stop the clinical trials. Meanwhile, it was reported that inhibition of the γ-secretase could lead to skin problems in the mice (Li et al., 2007), and that FAD mutations are actually the loss of function mutations (Chávez-Gutiérrez et al., 2012). The molecular mechanism of γ-secretase activity showed that FAD mutations alter its proteolytic processing leading to an increase in the long toxic peptides $A\beta_{1-42}$ and a reduction of the shorter $A\beta_{1-37}$ species (Chávez-Gutiérrez et al., 2012). Nowadays, it is clear that the goal is to increase the processing with the identification of new γ-secretase modulators.

Although the tumorigenic potential of GSIs in the skin could be explained by the inhibition of the NOTCH pathway that plays a tumor suppressor role in mice (Li et al., 2007), the molecular mechanism why GSIs lead to a worsening cognitive scenario is still unclear and several hypotheses have been formulated.

NOTCH is expressed in some neuronal stem cells and inhibition of the NOTCH pathway could lead to neurogenesis problems, however, adult neurogenesis is quite impaired in older adults (Díaz-Moreno et al., 2013), so other protein pathways and players could be behind this phenotype. In this sense, some have suggested that the increased levels of APP-CTF (also called C99) by the use of GSIs could be behind this toxic effects (Checler et al., 2021). Mice with a deletion in the APH-1 component of the γ-secretase complex accumulate more than 10-fold the levels of the membrane-bound CTF fragments of several substrates and showed signs of cortical atrophy, neuronal loss, and gliosis (Acx et al., 2017). This was associated with non-AD neurodegeneration and probably cognitive decline, but APP-CTF seems not responsible, as deletion of APP did not rescue that phenotype (Acx et al., 2017). This could be explained by the fact that in addition to APP and NOTCH, γ-secretase has several other substrates and some of them could contribute to the toxic effects after γ-secretase inhibition.

One of these substrates is the p75 neurotrophin receptor ($p75^{NTR}$) (Kraemer et al., 2014). Two things make $p75^{NTR}$ a suspect, one is its implication in developmental neuronal cell death, and the second is its high expression in the basal forebrain cholinergic neurons. Cholinergic synapses are ubiquitous in the human central nervous system. Acetylcholine plays an important role in memory function and has been implicated in aging-related dementia. Cholinergic neurons densely innervate the hippocampus and the cortex, suggesting that cholinergic transmission is likely to be critically important for memory, learning, attention, and other brain functions. Based on this, the basal forebrain cholinergic neurons (BFCNs) constitute one important target to study the action of GSIs.

The proteolysis of $p75^{NTR}$ has been suggested important for its pro-apoptotic activity. Recently, the increased levels of the p75-CTF in hippocampal and cerebral neurons by the use of γ-secretase inhibitors showed an increase in cell death in those neuronal populations (Vicario et al., 2015). A similar result was described earlier in sensory neurons (Underwood et al., 2008). The consequence of the inhibition of the γ-secretase complex on the BFCNs was studied recently in our labratory (Franco et al., 2021). The outcome was quite different from hippocampal and cereberal neurons, in the sense that the inhibition of the γ-secretase with GSIs, although it increased the levels of p75-CTF, does not induce cell death of the BFCNs. In the search for the mechanism of this result, we choose the cell lines PC12 cells (that express both p75 and TrkA) and a specific clone PC12-nnr5 cells (that express p75 but not TrkA). We found that GSIs induced the cell death of PC12nnr5 cells, and not PC12, indicating that TrkA activation may have a role in the survival of BFCNs. Actually when the primary cultures of BFCNs are incubated with GSIs plus a TrkA kinase inhibitor there was a significant cell death (Franco et al., 2021). This result suggests that the formation of p75-CTF *per se* is not indicative of a pro-death signal if TrkA is active, and that in the absence of TrkA signaling, p75-CTF is able to trigger cell death. The molecular mechanism proposed suggests that in the presence of GSIs, the levels of p75-CTF increased at the plasma membrane inducing its oligomerization and causing cell death by activation of TRAF6 and JNK/p38 pathways (**Frgure 1**). Finally we showed that TrkA induced an increase in the internalization of p75-CTF and a reduction of p75-CTF oligomerization at the plasma membrane, proposing this as the main mechanism by which TrkA protects cholinergic neurons from GSI-induced cell death.

How this could be related to the worsening of the cognitive decline in AD patients treated with GSIs? Although in aging the levels of p75 do not increase,

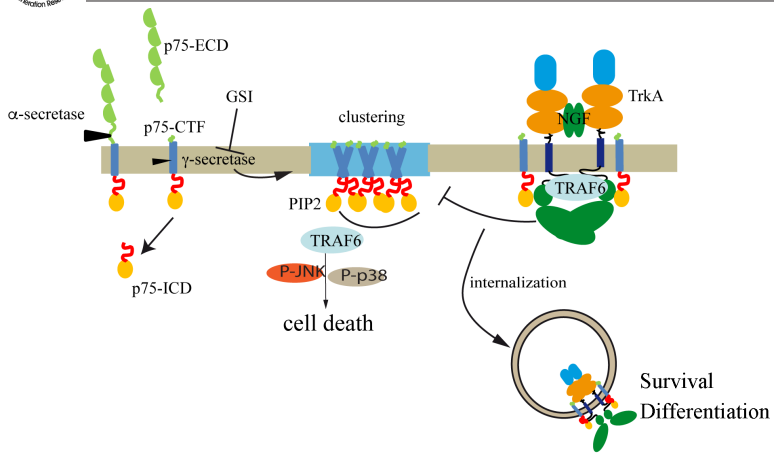

Figure 1 | **Model of the mechanism of p75-CTF induced cell death.**
Upon γ-secretase dysfunction, oligomerization of p75-CTF takes place in the cholesterol-rich region of the plasma membrane. Oligomers of p75-CTF induce the activation of caspase-3 cleavage and cell death in a mechanism dependent of TRAF6, JNK, and p38. TrkA kinase activity inhibits p75-CTF clustering and protects from cell death in part by the formation of a complex with p75-CTF and promoting its internalization. GSI: γ-Secretase inhibitor; JNK: c-jun N-terminal kinase; NGF: nerve growth factor; p75-CTF: p75 c-terminal fragment; p75-ICD: p75 intracellular domain; PIP2: phosphatidylinositol 4,5-biphosphate; TRAF6: TNF receptor associated factor 6.

the levels of TrkA decrease during healthy and pathological aging resulting in less pro-survival signaling (Ginsberg et al., 2006).

In summary, although other possibilities exist, the data presented in Franco et al. (2021) supports the role of p75 in a worsening of the cognitive decline in old AD patients treated with GSIs, suggesting that the use of NGF agonists potentiating the activation of TrkA, could alleviate or help in the treatment of degenerating diseases.

This work was supported by the Spanish Minister of Economy and Competitiveness grant SAF2017-84096-R and by the Generalitat Valenciana 2018-55, to MV.

Marçal Vilar*
Instituto de Biomedicina de València del CSIC, València, Spain
***Correspondence to:** Marçal Vilar, PhD, mvilar@ibv.csic.es.
https://orcid.org/0000-0002-9376-6544 (Marçal Vilar)

Date of submission: June 15, 2021
Date of decision: July 14, 2021
Date of acceptance: July 29, 2021
Date of web publication: December 10, 2021

https://doi.org/10.4103/1673-5374.330607
How to cite this article: *Vilar M (2022) Neurotrophin receptors, gamma-secretase inhibitors, and neurodegeneration of basal forebrain cholinergic neurons. Neural Regen Res 17(7):1493-1494.*

References

Acx H, Serneels L, Radaelli E, Muyldermans S, Vincke C, Pepermans E, Müller U, Chávez-Gutiérrez L, De Strooper B (2017) Inactivation of γ-secretases leads to accumulation of substrates and non-Alzheimer neurodegeneration. EMBO Mol Med 9:1088-1099.

Ancolio K, Marambaud P, Dauch P, Checler F (1997) Alpha-secretase-derived product of beta-amyloid precursor protein is decreased by presenilin 1 mutations linked to familial Alzheimer's disease. J Neurochem 69:2494-2499.

Chávez-Gutiérrez L, Bammens L, Benilova I, Vandersteen A, Benurwar M, Borgers M, Lismont S, Zhou L, Van Cleynenbreugel S, Esselmann H, Wiltfang J, Serneels L, Karran E, Gijsen H, Schymkowitz J, Rousseau F, Broersen K, De Strooper B (2012) The mechanism of γ-secretase dysfunction in familial Alzheimer disease. EMBO J 31:2261-2274.

Checler F, Afram E, Pardossi-Piquard R, Lauritzen I (2021) Is γ-secretase a beneficial inactivating enzyme of the toxic APP C-terminal fragment C99? J Biol Chem 296:100489.

Díaz-Moreno M, Hortigüela R, Gonçalves A, García-Carpio I, Manich G, García-Bermúdez E, Moreno-Estellés M, Eguiluz C, Vilaplana J, Pelegrí C, Vilar M, Mira H (2013) Aβ increases neural stem cell activity in senescence-accelerated SAMP8 mice. Neurobiol Aging 34:2623-2638.

Franco ML, García-Carpio I, Comaposada-Baró R, Escribano-Saiz JJ, Chávez-Gutiérrez L, Vilar M (2021) TrkA-mediated endocytosis of p75-CTF prevents cholinergic neuron death upon γ-secretase inhibition. Life Sci Alliance 4:e202000844.

Ginsberg SD, Che S, Wuu J, Counts SE, Mufson EJ (2006) Down regulation of trk but not p75NTR gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease. J Neurochem 97:475-487.

Kraemer BR, Yoon SO, Carter BD (2014) The biological functions and signaling mechanisms of the p75 neurotrophin receptor. Handb Exp Pharmacol 220:121-164.

Kuperstein I, Broersen K, Benilova I, Rozenski J, Jonckheere W, Debulpaep M, Vandersteen A, Segers-Nolten I, Van Der Werf K, Subramaniam V, Braeken D, Callewaert G, Bartic C, D'Hooge R, Martins IC, Rousseau F, Schymkowitz J, De Strooper B (2010) Neurotoxicity of Alzheimer's disease Aβ peptides is induced by small changes in the Aβ42 to Aβ40 ratio. EMBO J 29:3408-3420.

Li T, Wen H, Brayton C, Das P, Smithson LA, Fauq A, Fan X, Crain BJ, Price DL, Golde TE, Eberhart CG, Wong PC (2007) Epidermal growth factor receptor and notch pathways participate in the tumor suppressor function of gamma-secretase. J Biol Chem 282:32264-32273.

Underwood CK, Reid K, May LM, Bartlett PF, Coulson EJ (2008) Palmitoylation of the C-terminal fragment of p75(NTR) regulates death signaling and is required for subsequent cleavage by gamma-secretase. Mol Cell Neurosci 37:346-358.

Vicario A, Kisiswa L, Tann JY, Kelly CE, Ibáñez CF (2015) Neuron-type-specific signaling by the p75NTR death receptor is regulated by differential proteolytic cleavage. J Cell Sci 128:1507-1517.

C-Editors: Zhao M, Liu WJ, Li JY; T-Editor: Jia Y