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Transmembrane interaction motifs are driving the functional assembling of the γ-secretase complex

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Objectives:
The γ-secretase operates the intramembranous cleavage of amyloid precursor protein (APP), leading to amyloid beta (Aβ) release. Presenilin (PS1 or PS2) forms the catalytic core of this tetrameric complex. The functional assembling of the γ-secretase is highly coordinated: it relies on the proper interaction of the 19 transmembrane domains (TMD) belonging to the 4 protein members. Both PS contain two conserved GxxxG-like motifs in their TMD8, which play a key role in TM helix association. Our aim is to investigate the role of these motifs in the maturation of the γ-secretase.

Methods:
Presenilin GxxxG-like motifs were mutated by site-specific mutagenesis. Transient or stable overexpression of mutated PS were performed in CHO cells and MEP-PS knockout cells. Western blotting was used to measure the effect of mutations on PS maturation and APP metabolism. Gamma-secretase enzymatic activity was evaluated by a specific cell free assay. Aβ production was monitored by ECLIA. Assembling of the γ-secretase was analyzed by co-immunoprecipitation and Blue Native-PAGE.

Results:
In CHO cells, mutated PS were not correctly maturated (endoproteolysis defect). Mutations impaired γ-secretase activity. APP metabolism and Aβ production (Aβ40 and Aβ42) were affected in cells expressing PS1 mutants. Interestingly, mutated PS modified the migration profile of γ-secretase (BN-PAGE), suggesting a critical involvement of these motif in γ-secretase assembling. Rescued MEF-PSDKO are currently analyzed with the same techniques.

Conclusion:
The GxxxG-like motifs of the TMD8 of the Presenilin are fundamental for the maturation and assembling of the γ-secretase complex, and thus, for its activity.

Key words: Gamma-secretase complex, Presenilin, GxxxG-like motif, transmembrane interaction, mutagenesis, gamma-secretase maturation, complex assembly.