Generation of a novel murine model of Aβ deposition based on the expression of human wild-type amyloid precursor protein gene

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Mouse models of Alzheimer disease (AD) have been generated based on Amyloid-β Precursor Protein (AβPP) and the Presenilin (PSEN) gene mutations associated with familial AD (FAD). Such models have provided valuable insights into AD pathogenesis and represent an important research tool for the discovery of potential treatments. To model amyloid deposition in AD, we generated a new mouse line based on the presence of two copies of the genomic region encoding human wild-type AβPP as well as a mutation (L166P) in the murine Psen1. By ~6 months of age, these mice have begun to develop cerebral Aβ pathology with a significant increase in the levels of AβPP C-terminal fragments and Aβ42, as well as increase Aβ42/Aβ40 ratio. Since in the brain and other tissues of these mice, wild-type human AβPP mRNA and protein levels are comparable to those of endogenous AβPP, this model may allow studies about the role of AβPP isoforms in the pathogenesis of AD. This animal model may be suitable to test drugs aimed at inhibiting expression or altering splicing and processing of AβPP, without artifacts associated with the presence of mutations in AβPP or overexpression due to the use of exogenous promoters. These features of the new model are of critical importance in assessing the success of therapeutic interventions.

The neuropathologic hallmarks of Alzheimer disease (AD) are the accumulation of the insoluble 4 kDa amyloid-β (Aβ) peptide in brain parenchyma and vessel walls, the intraneuronal accumulation of neurofibrillary tangles (NFTs) composed of tau paired helical filaments (PHFs), and the extensive neuronal loss. Aβ peptides are generated in the “amyloidogenic pathway” by the proteolysis of the amyloid-β precursor protein (AβPP) by the β-site AβPP-cleaving enzyme 1 (BACE1) and the γ-secretase complex. Cleavage by α-secretase (ADAM, a disintegrin and metalloproteinase) and γ-secretase in the “non-amyloidogenic pathway” prevents Aβ generation. Missense mutations in AβPP located at or near the sites of proteolysis by β- and γ-secretase have been used to develop mouse models of AD. The mouse lines most frequently used express a mutation at the β-secretase cleavage site (Swedish double mutation, AβPP K670N/M671L) or a combination of the Swedish double mutation with a mutation in the γ-secretase site or within the Aβ sequence. There are only a few animal models based on the expression of a human AβPP sequence without mutations. In these models, high levels of expression were obtained by using strong exogenous promoters without significant amyloid deposition. Our laboratory recently reported amyloid deposition in mice expressing the entire wild-type (WT) AβPP gene using its endogenous regulatory elements.

Amyloid Deposition in Mice Expressing Human Wild-Type AβPP Gene

To further understand the mechanism(s) involved in Aβ generation and deposition associated with Presenilin 1 (PSEN1)
mutations, we generated a novel knock-in model based on a Leu to Pro mutation at codon 166 in the murine Psen1. This mutation was chosen since it is one of the most aggressive familial AD (FAD) mutations identified so far, originally found in a patient with onset of AD at 24 years of age. Clinically, the disease was characterized by progressive ataxia and spasticity soon followed by cognitive deficits. Neuropathologic findings included neutritic and cotton wool plaques, and cerebral amyloid angiopathy (CAA), as well as the presence of NFTs and severe neuronal loss. In addition, pyramidal tract degeneration and severe cerebellar Aβ deposits were present (Boyer et al., manuscript in preparation). The PSEN1 L166P mutation leads to a significant increase in Aβ production in cell culture, and very severe amyloid deposition in a double transgenic mouse model. However, Psen1-L166P knock-in mice do not develop plaque deposits, with mice analyzed up to 24 mo of age. The lack of amyloid deposition in the Psen1-L166P knock-in model may be in part due to differences in AβPP processing between mice and humans, and/or the three amino acids difference between the murine and the human Aβ seq.

Enhanced Production of Aβ42 Peptides Drives Amyloid Deposition

Amyloid deposition was not observed in heterozygous APP YAC x Psen1-L166P+/− or in APP YAC x Psen1-L166P−/− mice at the oldest age analyzed (24 months). However, replacement of the WT Psen1 allele led to amyloid deposition in APP YAC x Psen1-L166P+/−, in agreement with previous work done with Psen1-M146V knock-in mice where a reduction of γ-secretase activity rather than an increase in Aβ42 levels was proposed to drive amyloid deposition in the model. As in other animal models, the presence of a Psen1 knock-in mutation seems to enhance amyloidogenic processing of AβPP. Analysis of Aβ40 and Aβ42 levels in mouse brain by ELISA showed that in APP YAC x Psen1-L166P−/− mice, the replacement of one WT allele for a mutant allele led to a significant increase in Aβ42 levels, a significant reduction in Aβ40 levels and a significant increase in Aβ42/Aβ40 ratios in the neocortex and the hippocampus. The increase in levels of Aβ42 and the decrease in those of Aβ40 were high in APP YAC x Psen1-L166P−/− mice, and seemed to correlate with the number of WT Psen1 alleles replaced by the mutant L166P allele. In comparing with APP YAC x Psen1-L166P−/− mice, we did not observe a significant increase in total Aβ (Aβ40 + Aβ42) in APP YAC x Psen1-L166P−/− mice but it was significant (between 2 and 2.5 times) in APP YAC x Psen1-L166P−/− mice. A detailed biochemical analysis aimed at identifying...

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and characterizing the Aβ peptide species present in APP YAC x Psen1-L166P (+/+ ) mice, including post-translational modifications such as pyroglutamyl cyclization and N-terminal degradation which produces more hydrophobic Aβ species in humans18,21 is currently in progress.

γ-Secratease Processing and the Psen1-L166P Mutation

In the amyloidogenic pathway, AβPP undergoes successive proteolysis by BACE1 and the γ-secretase complex, while in the non-amyloidogenic pathway by α-secretase and γ-secretase.2,3 After α- or β-cleavage, the carboxyl terminal fragments (CTFs) of AβPP known as CTγx and CTβF, respectively, remain membrane-associated and are further cleaved by γ-secretase, a protease complex comprised of presenilin, nicastrin, anterior pharynx defective 1 and PS enhancer 2.1,4 Levels of CTFs seem to inversely correlate with total γ-secretase activity.22 Western blot analysis of brain samples showed that while full-length lev-

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Conclusions

Transgenic mouse models have proven useful for modeling various aspects of Aβ pathology in AD. However, none of these models recapitulates all aspects of AD. Mostly used are transgenic mice expressing mutant AβPP under the control of a strong heterologous promoter, however this feature adds to the complexity of evaluating the pathogenic significance of transgene-induced abnormalities. To circumvent this complexity, we created a mouse model in which the mutated gene is under the control of its own promoter. In order to accomplish this, we used gene targeting to modify the murine Pten1 gene on a transgenic mouse carrying two copies of the entire human WT AβPP gene. The latter possessed all the transcriptional regulatory elements required for proper spatial and temporal expression of AβPP. In the new mice (APP YAC x Psen1-L166P), expression of the Psen1-L166P mutation at normal levels under its endogenous control mechanism has a significant effect on AβPP processing and Aβ deposition. The APP YAC x Psen1-L166P model demonstrates that neither a strong promoter nor mutations in AβPP are needed to drive amyloid pathology. This model may be a unique tool, which might be ideal to explore a possible relationship between abnormal expression or splicing of AβPP and Aβ deposition. Moreover, it may be particularly suitable for testing therapies aimed at modifying secretase cleavage of AβPP in a cellular environment which is not affected by artifacts due to the presence of amino acid variations in the AβPP sequence. Such a difference may be of critical importance to assess therapeutic strategies for AD.

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