**Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders**

## Project: Identification of Genes Involved in Neuroprotection from Alzheimer Disease

**Claudia Prinzen, Rolf Postina & Falk Fahrenholz - Johannes Gutenberg University, Mainz - bio.chemie@uni-mainz.de**

## Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder, characterized by the formation of amyloid peptides (Aβ) and their deposition in the brain as senile plaques (1). The amyloid peptides Aβ40 and Aβ42, and especially their oligomeric aggregates, are believed to play a crucial and early role in the pathogenesis of AD by causing neurotoxicity (2), development of neurofibrillary tangles (3), impairment of long–term potentiation (LTP) (4), and age-related cognitive deficits (5).

Strategies to treat AD are aimed to prevent the formation of Aβ peptides and their deposition in the brain. Therefore, β- and γ-secretases that generate Aβ peptides by sequential cleavage of the amyloid precursor protein (APP) are obvious and main targets for the development of specific inhibitors (6).

Alternatively, increasing α-secretase activity in the brain provides an attractive strategy, since proteolysis of APP within the Aβ sequence precludes the formation of Aβ peptides (7, 8, 9). In addition, α-secretase cleavage of APP releases its N-terminal extracellular domain of about 110 kDa referred to as APPsα, which has neurotrophic and neuroprotective properties (10, 11) and enhances LTP (12). In behavioral paradigms, APPsα was demonstrated to enhance memory in normal and amnesic mice (13). In this respect it is interesting to note that a reduction of APPsα is evident in the cerebrospinal fluid of AD patients (14, 15).

Studies with neuronal cell lines and hippocampal neurons have shown exogenous APPsα, after binding to a yet unknown receptor, to activate several signal pathways which regulate calcium homeostasis. Furthermore, it was demonstrated that APPsα counteracts the pro-apoptotic action of mutant presenilin-1 by activation of NF-κB (16). At present it is still unknown which genes are regulated by the action of APPsα, and therefore, which are responsible for its long-term neuroprotective action.

Proteinases of the ADAM family (a disintegrin and metalloproteinase) are the main candidates as physiologically relevant α-secretases. We identified ADAM10 to possess α-secretase activity *in vitro*, in cultured cells and in a transgenic mouse model. Purified ADAM10 cleaves APP-derived peptides at the main α-secretase processing site between position Lys16 and Leu17 of the Aβ region, and after overexpression in HEK293 cells an augmented α-secretase-mediated processing of APP is evident. Furthermore, overexpression of the catalytically inactive mutant E384A of ADAM10 inhibits generation of neurotrophic APPsα by interfering with endogenous α-secretase cleavage of APP (17).

By means of cytochemical studies the coordinated expression of APP, the β-secretase BACE1 and ADAM10 have been described for mouse and human brain, supporting the role of ADAM10 and BACE as authentic α- and β-secretases (18).

## Results/Project Status

By using a transgenic animal model for AD we demonstrated that moderate neuronal overexpression of ADAM10 in mice transgenic for human APP[V717I] reduces the formation of Aβ peptides and prevents their deposition in plaques (Fig. 1). Inhibition of endogenously mediated α-secretase processing of APP by expression of a dominant-negative acting ADAM10 mutant enhanced the number and size of amyloid plaques in the brains of double-transgenic mice coexpressing APP[V717I] (Fig. 1).

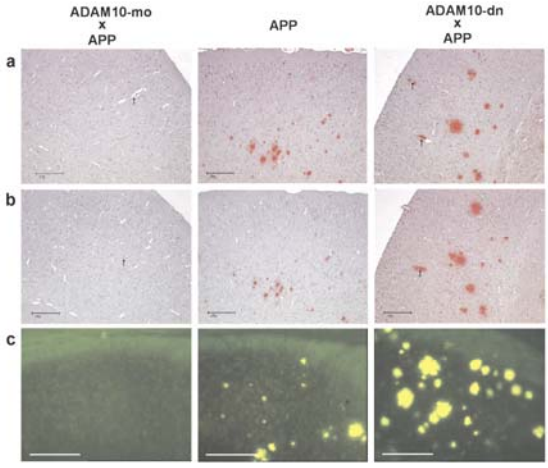


***Fig 1:*** *Detection and quantitation of amyloid plaques in brains of 17- to 19-month-old (A-D) APP[V717I] transgenic mice, double transgenic ADAM10-mo*APP[V717I] mice, and ADAM10-dn*APP[V717I] mice. Immunohistochemical detection of amyloid plaques in the neocortex in paraffin-embedded sections with either antibody 6F/3D (a) or antibody 4G8 (b).*
*Note that in ADAM10-mo* APP[V717I] mice no additional plaques were detected by antibody 4G8. Arrows point to blood vessels. Scale bars: 200 μm. (c) Thioflavine S-stained β-structures in the subiculum of 17- to 19-month-old mice; scale bars: 200 μm.*

Moreover, in double-transgenic ADAM10 x APP mice the amount of the C-terminal APP fragment generated by α-secretase is increased (19). This membrane-bound fragment is a substrate for subsequent γ-secretase cleavage that releases APPsα and the APP intracellular domain (AICD) which might serve as a potential transcriptional modulator. APPsα itself modulates the cytosolic calcium concentration, neutralizes pro-apoptotic factors, has neuroprotective properties and promotes neurite outgrowth, but the cellular target proteins of the APPsα action at present are unknown.

By overexpression of ADAM10 in the APP[V717I] background we observed significant improvements in cognitive abilities (Fig. 2). In comparison to APP[V717I] mice, long-term potentiation, learning and memory were improved in double-transgenic ADAM10 x APP[V717I] animals (19).

In contrast, a neuron-specific knock-out of the presenilin 1 γ-secretase prevented amyloid plaque formation, but did not improve cognitive deficits of APP[V717I] mice (20). Therefore, the beneficial effect of increased ADAM10 activity, including cognitive improvements, must be attributed to the combined effects of decreased levels of toxic Aβ peptides and increased amounts of neuroprotective APPsα. In this respect it is important to note that exogenously applied APPsα has been shown to enhance LTP in hippocampal slices (12), and in behavioral paradigms, intracerebroventricularly adiministered APPsα enhanced memory in normal and amnesic mice (13).

NGFN Nationales Genomforschungsnetz
GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

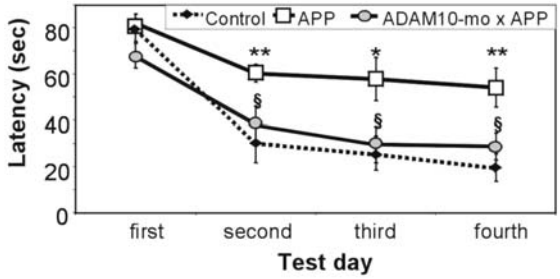


***Fig 2:*** *Acquisition of spatial learning in the Morris water-maze hidden-platform task. Learning deficit in APP[V717I] transgenic mice was ameliorated in double-transgenic ADAM10-mo*APP[V717I] mice. Lines represent mean ± SEM for 9–10 mice per group. *P < 0.05, **P < 0.01, APP[V717I] vs. control; §P < 0.05 APP[V717I] vs. ADAM10-mo x APP[V717I] by Student's t test.*

## Outlook

The project is aimed to identify genes mediating neuroprotective and neurotrophic functions of the α-secretase ADAM10. Activation of these genes thus might prevent Alzheimer disease and other neurodegenerative disorders.

Differentially expressed genes will be identified by applying Affymetrix mouse genome microarray analysis using brain samples of single-transgenic ADAM10, and dominant-negative ADAM10 overexpressing FVB/N mice. In these studies, non-transgenic FVB/N mice represent the control group. A direct target for APPsα action might be a putative APPsα receptor which may be upregulated in brains with very low amounts of APPsα, e. g. in dominant-negative ADAM10-expressing mice. Other direct target genes for the action of ADAM10 may comprise putative ADAM10 substrates such as the neuronal adhesion molecule L1. These might be upregulated during increased processing by ADAM10. Indirect target genes include those of cellular signaling molecules.

After identification of differentially expressed genes, the outcome of the microarray analysis shall be validated by quantitative real time RT-PCR and Western blots on mouse brain samples. Finally, putative ADAM10 target genes shall be knocked-down by small interfering RNAs in established cultured cell lines, and analyzed with regard to anti-apoptotic and anti-necrotic properties. In addition, the function of putative ADAM10 target genes will be analyzed after overexpression. New putative ADAM10 substrates can be validated by the use of specific ADAM10 inhibitors and by *in vitro* cleavage assays.

As the γ-secretase released C-terminus of APP (AICD) is a potential transcription factor, double-transgenic mice expressing either ADAM10 x APP or dominant-negative ADAM10 x APP will be analyzed for differentially expressed genes as described above. In ADAM10 x APP mice the amount of the C-terminal fragment of APP generated by the α-secretase is two-fold increased. Therefore, more AICD is likely to be released to modulated gene expression.

*Lit.: **1**. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001; 81(2):741-766. **2.** Geula C et al. Aging renders the brain vulnerable to amyloid beta-protein neurotoxicity. Nat Med 1998; 4(7):827-831. **3.** Gotz J et al. Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 2001; 293(5534):1491-1495. **4.** Walsh DM et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 2002; 416(6880):535-539. **5.** Chen G et al. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. Nature 2000; 408(6815):975-979. **6.** Vassar R, Citron M. Abeta-generating enzymes: recent advances in beta- and gamma-secretase research. Neuron 2000; 27(3):419-422. **7.** Sisodia SS. Beta-amyloid precursor protein cleavage by a membrane-bound protease. Proc Natl Acad Sci U S A 1992; 89(13):6075-6079. **8.** Haass C et al. Targeting of cell-surface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. Nature 1992; 357(6378):500-503. **9.** Esch FS et al. Cleavage of amyloid beta peptide during constitutive processing of its precursor. Science 1990; 248(4959):1122-1124. **10.** Small DH et al. A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. J Neurosci 1994; 14(4):2117-2127. **11.** Furukawa K et al. Activation of K+ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein. Nature 1996; 379(6560):74-78. **12.** Ishida A et al. Secreted form of beta-amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. Neuroreport 1997; 8(9-10):2133-2137. **13.** Meziane H et al. Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice. Proc Natl Acad Sci U S A 1998; 95(21):12683-12688. **14.** Lannfelt L et al. Decreased alpha-secretase-cleaved amyloid precursor protein as a diagnostic marker for Alzheimer's disease. Nat Med 1995; 1(8):829-832. **15.** Sennvik K et al. Levels of alpha- and beta-secretase cleaved amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients. Neurosci Lett 2000; 278(3):169-172. **16.** Guo Q et al. Secreted beta-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-kappaB and stabilization of calcium homeostasis. J Biol Chem 1998; 273(20):12341-12351. **17.** Lammich S et al. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc Natl Acad Sci U S A 1999; 96(7):3922-3927. **18.** Marcinkiewicz M, Seidah NG. Coordinated expression of beta-amyloid precursor protein and the putative beta-secretase BACE and alpha-secretase ADAM10 in mouse and human brain. J Neurochem 2000; 75(5):2133-2143. **19.** Postina R et al. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. J Clin Invest 2004; 113(10):1456-1464. **20.** Dewachter I et al. Neuronal deficiency of presenilin 1 inhibits amyloid plaque formation and corrects hippocampal long-term potentiation but not a cognitive defect of amyloid precursor protein [V717I] transgenic mice. J Neurosci 2002; 22(9):3445-3453.*