



Maladie d'Alzheimer et démences

Transgenic mouse models for Alzheimer's disease: the role of GSK-3 β in combined amyloid and tau-pathology

D. Muylaert, D. Terwel, P. Borghgraef, H. Devijver, I. Dewachter, F. Van Leuven

Experimental Genetics Group, LEGT-EGG, KULeuven, Campus Gasthuisberg, Leuven, Belgium.

SUMMARY

Describing and understanding the pathological processes which devastate the brain of Alzheimer's disease (AD) patients remains a major target for experimental biology. We approached this problem by generating different types of single and double transgenic mice that develop pathological hallmarks of AD. In APP-V717 mice, the progression from intracellular amyloid to diffuse and senile plaques with vascular deposits, is preceded by early defects in cognition and LTP. In Tau-P301L mice, the morbid tauopathy with intracellular filaments, cause mortality before age 1 year. Ageing APP-V717 \times Tau-P301L double tg mice (14-17 months) have combined AD-like pathology in hippocampus and cortex consisting of amyloid plaques and neurofibrillary tangles. Remarkably, while Tau-P301L mice die before age 1 year, the APP-V717 \times Tau-P301L double tg mice survive much longer, which correlates with alleviation of tauopathy in hindbrain, despite aggravation in forebrain. This hypothesis is corroborated in Tau-P301L \times GSK-3 β double transgenic mice, which have also an extended lifespan relative to Tau-P301L mice, that correlates with reduction of brainstem tauopathy. At the same time, Tau-P301L \times GSK-3 β mice have dramatic forebrain tauopathy, with "tangles in almost all neurons", although without hyper-phosphorylation of Tau. The data corroborate the hypothesis that GSK-3 β is the missing link between the amyloid and tau-pathology, and position GSK-3 β as prominent player in the pathogenesis in AD.

Keywords: Transgenic mice • Alzheimer's disease • GSK-3 β • Amyloid • Neurofibrillary tangles

RÉSUMÉ

Modèles de souris transgéniques de la maladie d'Alzheimer : rôle des GSK-3 en cas d'association des pathologies amyloïde et tau.

D. Muylaert, D. Terwel, P. Borghgraef, H. Devijver, I. Dewachter, F. Van Leuven, Rev Neurol (Paris) 2006; 162: 10, 903-907

Une meilleure description et compréhension des processus pathologiques dévastateurs pour le cerveau observés dans la maladie d'Alzheimer (MA) reste un objectif majeur de la biologie expérimentale. Notre approche du problème consiste en la création de différents types de souris transgéniques qui développent des signes de la MA. Dans la lignée de souris APP-V717, la progression d'un dépôt intracellulaire d'amyloïde vers la formation diffuse de plaques séniles et de dépôts vasculaires est précédée par des troubles précoces de cognition. Les souris Tau-P301L meurent de tauopathie avant l'âge d'un an avec dépôt de filaments intracellulaires. À l'âge de 14-17 mois, les souris transgéniques de la lignée APP-V717 \times Tau-P301L présentent une pathologie de type MA associant des plaques amyloïdes et des enchevêtrements neurofibrillaires à la fois au niveau de l'hippocampe et au niveau du cortex. Les souris Tau-P301L meurent avant l'âge d'un an tandis que les souris transgéniques APP-V717 \times Tau-P301L survivent plus longtemps, en corrélation avec une diminution de la tauopathie postérieure malgré une aggravation au niveau antérieur. De même les souris transgéniques Tau-P301L \times GSK-3 β survivent plus longtemps que les souris Tau-P301L, en corrélation avec une réduction de la tauopathie au niveau du tronc cérébral. Les souris Tau-P301L \times GSK-3 β présentent une tauopathie importante du tronc cérébral où presque tous les neurones sont pris dans des enchevêtrements neurofibrillaires sans qu'il y ait une hyper-phosphorylation du peptide tau. Ces données corroborent l'hypothèse que GSK-3 β est le lien manquant entre les pathologies amyloïde et tau, et que GSK-3 β joue un rôle prédominant dans la genèse de la MA.

Mots-clés : Souris transgéniques • Maladie d'Alzheimer • GSK-3 β • Amyloïde • Enchevêtrements neurofibrillaires

INTRODUCTION

The exact recapitulation and the molecular and cellular understanding of the pathological processes that wreck the brain of patients suffering from Alzheimer's disease (AD), remains a major target and challenge for experimental bio-

logists. We approach this problem by generating and characterizing different types of single and double transgenic mice that develop, either separately or in combination, the pathological hallmarks of AD, i.e. amyloid plaques and neurofibrillary tangles (Van Leuven, 2000 and references therein).

Correspondance : F. VAN LEUVEN, Experimental Genetics Group, LEGT-EGG, KULeuven, Campus Gasthuisberg ON1-06.602, B-3000 Leuven, Belgium. E-mail : Fred.VanLeuven@med.kuleuven.be

Over the last 15 years, our research group has generated ~65 independent knock-out and transgenic founder strains of mice. From these we selected and established transgenic mouse strains that are relevant models for different aspects of the amyloid and tau-pathology in AD. Moreover, we incorporated factors that intervene in the initiation, progression and termination of the neurodegeneration. The most relevant transgenic strains, designated by the wild-type or mutant human protein they express, are: APP-V717I, PS1-A246E, tau-4R, tau-P301L, GSK-3 β , neuronal or glial ApoE4, cdk5, p35, p25 besides mice with neuron-specific knock-out of PS1, i.e. PS1(n-/-) mice (*Table I*).

Most transgenic constructs are based on the engineered mouse thy1-gene promoter to express the transgene (i) exclusively in neurons and (ii) only post-natally (~P10 onwards).

Table I. – List of LEGT_EGG transgenic mice with most relevant references.

Liste des souris transgéniques LEGT-EGG ayant les référentiels les plus pertinents.

APP-V717I	Moechars D <i>et al.</i> , 1999
	Van Dorpe <i>et al.</i> , 2000
	Dewachter I <i>et al.</i> , 2000
	Willem M <i>et al.</i> , 2004
	Etcheberrigaray <i>et al.</i> , 2004
	Postina R <i>et al.</i> , 2004
PS1-A246E	Vanhouette G <i>et al.</i> , 2005
	Van Dooren T <i>et al.</i> , 2005
	Dewachter I <i>et al.</i> , 2000
PS1(-/-)	Van Dorpe <i>et al.</i> , 2000
	Schneider <i>et al.</i> , 2001
	De Strooper B <i>et al.</i> , 1998
tau-4R	Dewachter I <i>et al.</i> , 2002
	(conditional KO)
	Spittaels K <i>et al.</i> , 1999
tau-P301L	Terwel D <i>et al.</i> , 2005
	Vandebroek T <i>et al.</i> , 2005
	Boekhoorn K <i>et al.</i> , 2006
ApoE4 neuronal & glial	Terwel D <i>et al.</i> , 2005
	Tesseur I <i>et al.</i> , 2000 a
	Tesseur I <i>et al.</i> , 2000 b
GSK-3 β	Van Dooren T <i>et al.</i> , 2006
	Spittaels K <i>et al.</i> , 2000
	Tilleman K <i>et al.</i> , 2002
	Terwel D <i>et al.</i> , 2002
cdk5/p35	Spittaels K <i>et al.</i> , 2002
	Van Den Haute C <i>et al.</i> , 2001

Both characteristics are very important, because we want to study adult and ageing mice and must therefore avoid interference with embryonal development (Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000; Tesseur *et al.*, 2000a, b; Dewachter *et al.*, 2002). Transgenic mice are primarily maintained in the FVB/N strain to avoid problems with mixed genetic backgrounds during cross-breeding into multiple transgenic strains. All experimental transgenic mice are genotyped by specific PCR routinely on two different occasions during the course of their life-time, and all historical and analytical data are kept on individual files.

For extensive characterization of most of these mice, we refer to our publications since here we can and will only briefly review the most salient points of the most relevant models for AD. In addition we include some of our unpublished observations, either work in progress or in preparation or submitted, which will be discussed at the Paris meeting.

APP-V717I transgenic mice

Our APP-V717I transgenic mice develop progressively and robustly the typical amyloid pathology of AD, including sequentially (i) intracellular amyloid peptides in a vesicular appearance (age 3-9 months), (ii) diffuse plaques (10-12 months) followed by (iii) senile amyloid plaques in brain parenchym (12-15 months) and (iii) finally cerebrovascular angiopathy (15-18 months) all further progressing with age. Thereby, the APP-V717I mice are an excellent model for the amyloid pathology in AD (Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000; Dewachter *et al.*, 2000).

The APP-V717I transgenic mice serve as valuable pre-clinical models for the amyloid pathology in AD and are continuously further characterized and exploited in many academic collaborations and in projects with industrial partners for biotechnological and pharmaceutical drug development.

Modulation of amyloid pathology by α -, β -, and γ -secretases, i.e. ADAM10, BACE1 and PS1

The amyloid pathology and the early cognitive defects are modified positively by co-expression of ADAM10, and thereby proven the most important α -secretase in vivo (Postina *et al.*, 2004). We put forward this proteinase as a very worthwhile therapeutic target in AD, although its mechanisms of action and activation are not fully understood, presenting both fundamental and pharmacological problems — to be resolved by further studies.

On the other hand, BACE1 aggravated the amyloid pathology in brain parenchym, but surprisingly this happened at the expense of the vascular amyloid pathology which was nearly completely absent in APP-V717IxBACE1 double transgenic mice (Willem *et al.*, 2004). The underlying biochemical reason was proposed, and confirmed most

recently, by the extra cleavage by BACE1 between positions 10 and 11 of the amyloid sequence, yielding a dramatic increase in N-truncated amyloid peptides A β 11-42 and of its N-pyro-glutamylated derivative (Willem *et al.*, 2004; Van Dooren *et al.*, 2006). These N-truncated peptides are evidently much less soluble than the full-length A β 42 and will therefore precipitate closer to their origin of synthesis, i.e. the neurons. Thereby they cause not only a dramatic 5 to 10-fold increase in parenchym amyloid deposits but moreover hamper diffusion of other amyloid peptides to the perivascular space, and thereby prevent CAA formation (Willem *et al.*, 2004; Van Dooren *et al.*, 2006; Weller, 1998).

The clinical mutant PS1-A246E, responsible for early onset familial AD, does not cause an apparent phenotype on its own in transgenic mice (Van Dorpe *et al.*, 2000; Dewachter *et al.*, 2000). However, in combination with human mutant APP, all aspects of the amyloid pathology are dramatically aggravated in APP-V717I \times PS1-A246E double transgenic mice (Van Dorpe *et al.*, 2000). The underlying mechanism is nevertheless different from that induced by aging (Dewachter *et al.*, 2000). Besides increasing the production of amyloid peptides, mainly of A β 42, concomitant with decreasing production of A β 40, the mutant PS1 also disturbed the neuronal calcium homeostasis (Schneider *et al.*, 2001; Herms *et al.*, 2003; Ris *et al.*, 2003; Dewachter *et al.*, 2006). This finding constitutes an extra contribution to the pathology, and although the underlying mechanism is not yet understood, it explains the severe neurodegeneration in familial AD cases due to PS1 mutations.

On the other hand, neuronal deficiency of PS1 by a neuron specific knock-out implemented by LoxP-Cre recombinase approach, lowers the production of amyloid peptides about 3-4 fold, as expected. Thereby the amyloid pathology in terms of amyloid plaques and CAA was completely prevented, but surprisingly, not the cognitive decline of APP-V717I \times PS1(n-/-) (Dewachter *et al.*, 2002, 2006), more recently independently confirmed (Saura *et al.*, 2005). Obviously, not the amyloid plaques, but soluble amyloid peptide oligomers, as well as their accumulated precursors, the C99 (β -CTF) fragments of APP, are to blame for the "synapto-toxicity" (Dewachter *et al.*, 2002; Dewachter and Van Leuven, 2002).

No tauopathy in APP-V717I mice or its double or triple transgenic derivatives

Despite their robust amyloid pathology, our APP-V717I transgenic mice do not develop extensive tau-pathology, which is not different from any and all similar amyloid models. Only dystrophic neurites in and around the senile amyloid plaques in old APP-V717I mice contain some AD-specific tau-epitopes like AT8 and AT100 (Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000; Muyliaert *et al.*, 2006). The model must therefore be further adapted by crossing with other transgenic mouse strains, like the FTD-mutant tau-P301L strain (Terwel *et al.*, 2005; Muyliaert *et al.*, 2006) but also with GSK-3 β [S9A] (Spittaels *et al.*, 2000).

Different Tau-4R transgenic mice and different effects of GSK-3 β and cdk5

Tau-4R mice that express the longest human wild-type isoform of tau, develop a dramatic axonopathy with severe motor problems (Spittaels *et al.*, 1999). The axonopathy is typified by axonal dilatations ("balloons") due to blockage of axonal transport over the microtubular tracks, due to excessive binding of tau-4R to the microtubules (Spittaels *et al.*, 1999, 2000; Kunzi *et al.*, 2002). The axonopathy and motor problems disappeared completely by co-expression of GSK-3 β (Spittaels *et al.*, 2000) but not by cdk5/p35 (Van den Haute *et al.*, 2001) demonstrating that in vivo phosphorylation of tau by GSK-3 β , but not by cdk5, is physiologically important in reducing the binding of tau-4R to MT and thereby to control integrity of axonal transport (for review see Terwel *et al.*, 2002 and references therein).

Tau-P301L mice develop a moribund tauopathy with hyperphosphorylation and aggregation of protein tau, resulting in neurofibrillary tangles (NFT) and neurodegeneration that is lethal around the age of 10-12 months (Terwel *et al.*, 2005). Tau-P301L mice thereby recapitulate the disease process of FTD patients. Remarkably, at young age the tau-P301L mice show better cognitive performance and stronger LTP than age-matched non-transgenic mice (Boekhoorn *et al.*, 2006). This is construed to support the hypothesis that not the mutant itself, but its (unknown) impact on the phosphorylation of mutant tau with ageing causes the moribund tauopathy.

The demonstration that cdk5 was not very effective as tau-kinase in vivo in triple tau-4R/cdk5/p35 transgenic mice (Van den Haute *et al.*, 2001) conflicted with the notion that cdk5 was identified as tau kinase II. The demonstration of increased phosphorylation of tau and neurofilaments in p35-/- mice (Hallows *et al.*, 2003) fuses seamless with our observations in yeast cells that not hyper- but hypo-activity of cdk5 causes hyper-phosphorylation, conformation and aggregation of protein tau (Vandebroek *et al.*, 2005, 2006).

Combined pathology in APP-V717I \times Tau-P301L double tg mice and contribution of GSK-3 β

Combined amyloid and tau-pathology as a more complete model for AD is obtained in APP-V717I \times Tau-P301L double transgenic mice. Offspring of sufficient old age (14-18 months) contain amyloid and tau-pathology in all important brain regions, including hippocampus and cortex. Moreover, in addition to the combined pathology, synergistic interaction was obvious by the more intense and more widespread tauopathy in double transgenic mice, relative to the parental strains. Brain regions that are typically subject to pathology in AD, i.e. entorhinal cortex, hippocampus and neocortex, were loaded with amyloid and NFT in aged APP-V717I \times Tau-P301L double transgenic mice. Ongoing analysis concerns the earliest symptoms of cognitive, social and sensori-motor behavior, to define the exact nature of the synergism in pathology and its repercussions on, and

correlation with, the clinical symptoms (Muyllaert, Terwel, unpublished data).

Even more dramatic was the tauopathy observed in aging Tau-P301L×GSK-3β[S9A] double transgenic mice, yielding biochemical evidence for very intense hyper-phosphorylation of protein tau relative to the parental Tau-P301L mice — as expected. A dramatic increase in the number of neurons loaded with NFT is evident in all cortical and hippocampal neurons in aged double transgenic mice (14-18 months), which become literally packed with filamentous tangles “in all neurons”. While these data underline that GSK-3β is the most potent tau-kinase *in vivo*, the unexpected finding that the double transgenic mice survive longer than the parental tau-P301L mice is highly reminiscent of the rescued axonopathy in Tau-4R×GSK-3β mice (Spittaels *et al.*, 2000) and remains to be explained experimentally.

Ageing APP-V717I×tau-P301L double tg mice (14-18 months) have combined AD-like pathology in hippocampus and cortex consisting of amyloid plaques and neurofibrillary tangles. Amyloid pathology is similar in aspect, but more extensive than in age-matched APP-V717 mice. The tau-pathology is dramatically enhanced in the forebrain of APP-V717I×Tau-P301L double transgenic mice. Remarkably, while tau-P301L mice die before age 1 year, the APP×Tau double tg mice survive, which appears due to alleviation of the prominent brainstem tauopathy. Even more remarkable, this hypothesis is corroborated in tau-P301L×GSK-3β double tg mice since their extended lifespan (relative to P301L mice) also correlates with dramatic reduction in brainstem tauopathy. At the same time, tau-P301L×GSK-3β double transgenic mice have a dramatic forebrain tauopathy, with “*tangles in almost all neurons*” in hippocampus and neo-cortex. Differential phosphorylation of mutant tau-P301L by GSK-3β does not result in an obvious initial hyper-phosphorylation of soluble protein tau, but does eventually lead to accumulation of hyper-phosphorylated tau as insoluble fibrils and tangles. The data corroborate the hypothesis that GSK-3β is the missing link between the amyloid and tau-pathology in AD brain. Despite the tauopathy in both APP-V717I×Tau-P301L and GSK-3β×Tau-P301L double tg mice, axonopathy was absent and only prominent in our tau-4R mice (Spittaels *et al.*, 1999), from which it also can be rescued by co-expression of GSK-3β (Spittaels *et al.*, 2000). Clearly, GSK-3β is becoming more and more important as central player in the pathogenesis in AD, linking the amyloid and tau pathology.

In conclusion, our transgenic mouse models for the different aspects of the molecular problems in AD, continue to define novel characteristics that were unexpected when we started this exercise more than a decade ago. Not only became the early defects that precede the amyloid plaques, the basis for a further adaptation of the amyloid cascade hypothesis, which centers today on the “synaptotoxic action” of amyloid peptide oligomers, be it trimers, tetramers or dodecamers. In addition, the animal models allowed us to define important functions of the three major secretases *in vivo*, i.e. (i) PS1 with different contributions of wild-type

and mutant PS1 as γ -secretase or in calcium ion homeostasis, (ii) BACE1 as β -secretase in controlling the balance between parenchymal and vascular amyloid, and last but not least (iii) ADAM10 as the important non-amyloidogenic α -secretase *in vivo*, confirmed as additional and attractive therapeutic target for Alzheimer’s disease.

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