TRANSGENIC MOUSE MODELS OF ALZHEIMER DISEASE

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GENERAL PRINCIPLES

Transgenic models of Alzheimer disease (AD) continue to gain credibility as more features of the human disease are shown to be represented in the mice. Tau pathology and extensive cell loss are still not seen, however. Despite these shortcomings, the mice are excellent models of amyloidosis, and this field of study has been highly informative in advancing our understanding of *in vivo* responses to amyloid insult and the mechanism by which genetic lesions may cause AD. Because many investigators believe that amyloid (or its precursors) is critical to initiation of the disease, the mice are now being used to test therapeutic agents that may have utility in patients with AD. In addition, new models that address the issue of tau pathogenesis have been created that may help to explain the relative contribution of tau and amyloid to the pathogenesis of AD.

AD is a progressive neurodegenerative disease. Most cases of AD occur sporadically, but familial forms of the disease have been most widely studied because of the insight they give into disease origin. Genetic causes of the disease are heterogeneous and include mutations or variants in several genes including the amyloid precursor protein *(APP)* gene, the presenilins *(PS),* and apolipoprotein E *(APOE)* (reviewed in ref. 1). The disease phenotype is remarkably consistent and includes the accumulation of β-amyloid (Aβ) and its deposition into senile plaques, the formation of tau-containing tangles, reactive gliosis, inflammation and an immune response, neurodegeneration, cholinergic deficit, and cognitive impairment.

Report of the first transgenic mouse to develop a robust AD-related phenotype was published in 1995 by the Exemplar/Athena Neuroscience group (2). This line (known as PDAPP) overexpresses mutant *APP* at high enough levels to generate sufficient Aβ for extracellular deposits (plaques) to form in relevant regions of the brain. In 1996, a second line (Tg2576), created by Karen Hsiao and colleagues, also

made sufficient amyloid for deposits to form, and, in addition, this mouse showed age-related cognitive impairment (3). Subsequently, other cDNA mice (4,5) and mice overexpressing genomic constructs (6) have also been shown to form amyloid in old age. Several research groups have created transgenic mice that overexpress mutant presenilin (7–9), but these mice do not show amyloid deposition, most likely because they have insufficient levels of the Aβ peptide.

Most of the current work on the mice focuses on cellular response to amyloid accumulation and its relevance to AD. Recently, the PDAPP line of mice was used to test the feasibility of modulating amyloid levels by immunization with Aβ (10). The results of this experiment suggested that amyloid modulation is indeed possible and that some of the secondary effects of amyloidosis (gliosis and neuritic changes) can be prevented. This work opens up a new direction in amyloid research and may well have significant impact on the development of human therapies.

RECENT ADVANCES IN PHENOTYPE ASSESSMENT IN TRANSGENIC MODELS OF AD

Amyloidosis

Several studies aimed to modulate the amyloid phenotype by crossing in other transgenes such as *PS1* or *TGF*-β (transforming growth factor-β). The studies showed that when a *PS1* mutant mouse was crossed to an *APP* overexpressing mouse, the levels of Aβ42 (43) were increased in the double transgenic mice, and this elevation had a profound influence on the age at which amyloid deposition could first be detected (6,11,12). In one cross, the age at which amyloid deposits were first identified was reduced from 9 to 12 months to 10 to 12 weeks (13) which is the earliest age at which amyloid has been reported. When *TGF*-β cDNA mice were crossed into an *APP* overexpressing line, amyloid deposition was again accelerated, and deposition was far more prominent in the vasculature (14). Apart from show-

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ing that these pathways interact, one outcome of the crossed-mouse studies is to enhance, and in some cases to modify, the phenotype, thus providing us with better models.

Presenilin Transgenics

In terms of the amyloid phenotype seen in AD, the most significant phenotype in the mutant *PS* transgenics continues to be the specific elevation of Aβ1-42(43) (7–9). Mutant *PS2* transgenics have been created that also show elevation in Aβ1-42(43) (15), a finding that strengthens the argument that APP and the presenilins interact, either directly, as suggested by Wolfe et al. (16), or indirectly, and *PS* mutations cause AD through APP/Aβ modulation. More recently, several studies on cDNA and targeted knock-in *PS1* mice have shown that mutant *PS* mice show deficits in calcium homeostasis (17–19) and, in some models, impaired mitochondrial function (17).

Studies of knockout *PS1* animals have shown that PS1 plays an important role in development, because lack of the protein leads to a deficiency in somitogenesis during early embryogenesis that results in severe skeletal abnormalities and prenatal death (20,21). These abnormalities strongly resemble those seen in Notch knockout mice (22), and this observation, coupled with several studies *in vivo* and *in vitro,* suggests that Notch and PS1 interact in some way to affect normal cellular function that may downstream affect signaling, differentiation, and development.

PS1 has been strongly implicated in other signaling pathways because several potential components of signal transduction pathways have been identified as presenilin-interacting proteins. These include several proteins containing an armadillo repeat region, which is a 42 amino acid motif that has been identified in proteins involved in cell–cell adhesion, protein–protein interaction, and signal transduction. The best known of these is β-catenin, and both β- and its homologue, δ-catenin, have been shown to interact with PS1 (23). The effect of *PS1* mutations on β-catenin stability and hence its downstream effects are controversial. In transgenic mice and human familial AD brain homogenates, for example, mutations in PS1 are linked to increased degradation of β-catenin (24), whereas other studies have suggested no effect or increased activity for the wild-type and mutant PS1 protein (25–27). Quite how *PS1* mutations may lead to AD through a β-catenin pathway is unclear, although in addition to the effect on Aβ42, one action maybe on the action of GSK-β and hence tau phosphorylation (for a review, see Alzheimer forum panel discussion at *www.alzforum.org/members/forums/journals/catenin/index. html*).

Neurodegeneration

Human AD brain shows extensive neurodegeneration, both in cholinergic neurons of the nucleus basalis (28) and in noncholinergic neurons throughout the cortex and hippocampus. Studies showed that fibrillar Aβ peptides are toxic to neurons in culture (29), and the overproduction of human Aβ in the brains of transgenic mice was therefore expected to cause extensive neurodegeneration. Several of the best characterized mouse models were examined for overt cell loss (30–32), but PDAPP, Tg2576, and the Tg2576/PS1 cross-mouse did not show significant cell loss, even though the amyloid burden exceeded 30%. One model was reported to show significant cell loss, but only in the hippocampus (32). Although overt cell loss is not seen in mice such as Tg2576/PS1, neurites in close proximity to amyloid deposits are severely dystrophic, and cellular disturbance is rife, as shown by extensive lysosomal activation (unpublished data). In addition, magnetic resonance imaging (MRI) revealed differences in the volume of structures such as the lateral ventricles and the corpus callosum between mice with and without amyloid, a finding that may reflect loss of neuropil, or shrinkage of cells rather than cell death *per se* (unpublished data). Advances in MRI in the study of human AD brain (33) suggest that it will soon be possible to image plaques directly *in vivo,* although contrasting agents may be required for mouse imaging because resolution is poor. Although the field is in its infancy, the application of functional and structural MRI to the analysis of models is predicted to have significant impact, especially because longitudinal analyses can be performed on the same mouse, including the effects of drug treatments.

Cholinergic Deficits

Although modulation of the cholinergic system has been a therapeutic treatment for AD dementia for many years, investigations into the response of cholinergic neurons to amyloid insult has only recently been studied in the transgenic mice. Immunohistochemical analysis has shown that cholinergic markers accumulate in swollen abnormal neurites around amyloid deposits in the cortex (4), and our own studies have shown that in the early stages of deposition, neurons in the nucleus basalis of depositing mice appear normal, but their projection areas in certain regions of the cortex show a significant reduction in synapse density and size (34). Significantly, this finding correlates with reduced cholinergic neurotransmitter activity (unpublished data). Further work to assess how cholinergic neurons in all areas of the brain respond to increasing amyloid burden and age is under way because cholinesterase inhibitors are currently considered to be valid therapeutic agents for AD.

Cognitive Impairments and Neurodegeneration

Recreating human cognitive impairment is perhaps the greatest challenge facing genetic engineers working on

transgenic models of human dementia. Mice are genetically less suitable to behavioral testing than rats because performance data on normal mice from different strains are often contradictory. Despite these reservations, most of the transgenic mice that form amyloid deposits have been tested for cognitive impairment. The PDAPP mouse (35,36), the Tg2576 mouse (3), and the Tg2576/PS1 cross-mouse (12) have all shown a deficit in tests of hippocampal dysfunction before amyloid deposits form, a finding that strongly suggests that overt amyloid accumulation or deposition is not responsible for this early cognitive impairment. Deficits in water maze performance that correlate with increasing age and amyloid burden and with decreasing long-term potentiation have been reported for Tg2576 mice (3,37).

Tau Pathology

One of the major deficits in the current AD mice is the lack of tau pathology. In humans, tau pathology takes the form of intracellular tangles of an abnormally phosphorylated form of the tau protein, which associates into paired helical filaments (PHFs). Both amyloid plaques and tau tangles are pathognomic features of human AD, and their relative contribution to the disease has long been disputed. The identification of AD-causing mutations in *APP* and the presenilin genes, however, adds weight to the amyloid-based hypothesis of pathogenesis, which assumes that tau abnormalities are a secondary lesion that form in response to amyloid accumulation. To examine this link, transgenic mice with extensive amyloid burden were examined for abnormal tau pathology by immunohistochemical analysis. This work showed that amyloid deposits in transgenic mice are ringed by dystrophic neurites that are immunoreactive for markers of phosphotau epitopes such as phosphoserine 202 (4,38). These epitopes are phosphorylated to some degree in normal brain, but they are hyperphosphorylated in AD brain. In the mice, it is not yet clear whether the immunoreactivity around deposits reflects local hyperphosphorylation of tau or simple elevation of tau protein levels in response to neuritic damage. In the human AD brain, subsets of neurons are also immunolabeled with antibodies to both tyrosine phosphotau and the signaling protein fyn, which is an src, nonreceptor tyrosine kinase (39). Co-IP has shown that the N-terminus of tau and the SH-3 domain of fyn interact directly, a finding suggesting that tau may be involved in signal transduction pathways (40). Interestingly, fyn binds another signaling protein, FAK (41), which is itself up-regulated by Aβ (42). Ongoing work includes a study of how FAK, fyn, tau, and Aβ interact in transgenic animals with and without elevated amyloid.

It is clear, however, that tau pathology does not develop further in the mutant *APP* and *PS* transgenic animals, a finding that suggests that either Aβ/amyloid accumulation is not detrimental to tau or differences between mouse and human brain preclude the formation of pathogenic tau, as suggested by Geula et al. (43). This suggestion has been countered by reports that mouse tau is capable of forming PHF-like structures *in vitro* (44). We have created a line of transgenic mice that overexpress all isoforms of human tau under the control of the human tau promoter (45), in an effort to "humanize" the tau environment in amyloid-depositing mice by cross-breeding the different lines. Unfortunately, even in the presence of extensive amyloid, the mice do not form neurofibrillary pathology (unpublished data), a finding suggesting that the mice are either still deficient in another human component or that Aβ accumulation does not stimulate tau pathogenesis, at least in mice.

Mutations in tau have been shown to cause frontal temporal lobe dementia (FTD-17) (46–48), which, in some cases, appears to result from an imbalance in tau isoform ratios (47). Transgenic mice that overexpress normal human tau isoforms and recreate this imbalance show a partly unexpected phenotype in that the mice develop hind limb weakness mainly resulting from spheroid accumulations composed of tau and neurofilament form, which are particularly prevalent in the axons of motor neurons (45,49,50). Although the tau does appear to be in an abnormal conformation, the relevance of the axopathy to human FTD-17 or the tauopathies is unclear. Mutant tau transgenic mice have been created, but their phenotype has not yet been described. It is likely that the creation of a battery of tau transgenic mice will provide long-awaited resources for the study of the normal and abnormal biology of this important cell component.

ACKNOWLEDGMENT

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