Relevance of Transgenic Mouse Models to Human Alzheimer Disease

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The most common cause of dementia, AD accounts for 60–70% of all dementia cases and afflicts >15 million individuals worldwide. The disorder is characterized by severe memory loss, with episodic memory being particularly impaired during the initial phases. At present, the disorder is not curable. Most AD cases occur sporadically (SAD), although inheritance of certain susceptibility genes enhances the risk. A small minority of AD cases (<1%) is inheritable (referred to as FAD) and is caused by mutations in genes encoding APP, PS1, or PS2.

Pathological Hallmarks of AD

Definitive diagnosis of AD occurs during post-mortem examination upon detection of two hallmark pathologies. The first is amyloid plaques, which consist of Aβ. The length of Aβ can vary, but a 42-amino acid variant is considered neurotoxic because of its propensity to readily aggregate into oligomers and fibrils. All mutations associated with FAD affect the aggregation and/or production of Aβ, which is sequentially cleaved from the APP holoprotein, first by an enzyme known as BACE (beta-APP-cleaving enzyme) and then by an enzymatic complex known as γ-secretase, in which the presenilins form the catalytic core (1). FAD-associated mutations in APP cluster around the β-secretase cleavage site (e.g. Swedish mutation), in key amino acids affecting its ability to aggregate (e.g. Arctic and Dutch mutations), or around the γ-secretase cleavage site, which increases production of the longer Aβ42 peptide (e.g. London mutation). PS mutations play a similar role by favoring production of Aβ42 at the expense of Aβ40. In vitro experiments and transgenic mice have shown us that the aggregation state of Aβ is crucial, that it can also accumulate intraneuronally, and that it can mediate a diverse range of pathological effects on cellular function (2).

The second pathological hallmark is the appearance of intraneuronal aggregates composed of the microtubule-associated protein tau. Hyperphosphorylation of tau leads it to dissociate from the microtubules and aggregate within the axoplasm as NFTs (3). Furthermore, tau dissociation leads to a reduction in microtubule stability and impaired axonal transport, ultimately leading to neuronal dysfunction and loss of synapses and subsequent retrograde degeneration (4). Mutations in the gene for tau (MAPT) are not associated with AD but cause FTDP-17 (frontal temporal dementia with parkinsonism 17), showing that disruption of tau function directly leads to neurodegeneration. Besides these two hallmark lesions, other reactive processes occur such as inflammation (5) and additional disturbances in cellular function through calcium dyshomeostasis (6) and oxidative stress (7), which cumulatively cause marked cortical and hippocampal neuronal and synaptic loss.

Modeling Amyloid Pathology in Mice

APP-overproducing mice develop amyloid deposits similar to those found in the human brain in an age-dependent fashion (supplemental Table 1). Despite chronic APP production, plaques typically accumulate in mid-to-late adulthood in the majority of these animals, although this is largely dependent on expression levels or the number of FAD mutations introduced. Notably, plaque formation is accelerated when the longer Aβ42 is preferentially cleaved from APP, as this peptide is more prone...
MINIREVIEW: Mouse Models of Alzheimer Disease

to aggregate than Aβ40 and leads to earlier and more severe cognitive decline (10). The importance of Aβ42 to disease progression was highlighted by showing that elevated levels of Aβ40, the shorter more common form of Aβ, actually prevented the formation of Aβ pathology in the widely used Tg2576 mouse model. On the contrary, elevated levels of Aβ42 markedly exacerbated pathology in the same mouse model (11).

Aβ plaques found in the brains of AD Tg mice appear structurally similar to those found in the human brain; they initiate as diffuse plaques consisting mainly of Aβ42, develop a dense Aβ42 core, and then incorporate Aβ40 as well as numerous other non-Aβ components such as ubiquitin and α-synuclein (12). Positron emission tomography (PET) imaging indicates that the radiotracer Pittsburgh Compound B binds to Aβ deposits in mice with less affinity than to human Aβ deposits, possibly due to the increased levels of pyroglutamate in human Aβ deposits (13). However, work in Tg mice has highlighted the dynamic nature of extracellular plaques and has also aided in the clarification of important elements in both the brain environment and the Aβ peptide needed for aggregation of Aβ into plaques. Although formation of plaques in AD Tg mice is typically age-dependent (as is AD pathology in humans), plaque formation occurs very quickly in the brains of older AD Tg mice. This has been shown by creating a “window” in the skulls of APP Tg mice (14) and further supported by data showing that plaque volume in aged AD Tg mice rapidly returns to high levels within 30 days following plaque removal by immunotherapy (15), in grafts of wild-type tissue into AD Tg mouse brains (16), and in the brains of pre-pathological AD Tg mice following injection with extracts from human AD brain or aged AD Tg mouse brain (17). These data indicate that the adult AD Tg mouse brain is ripe for the development of Aβ pathology, and the latter study also suggests that the ability of Aβ to act as a seed for aggregation is dependent on its source. Although some differences have been shown between Aβ pathology in human AD and mice overexpressing human APP (18), AD Tg mice develop Aβ plaques that are remarkably similar to those seen in humans.

The majority of AD Tg models exhibit memory impairments on various cognitive tests (19). Critically, cognitive deficits in AD Tg mice occur prior to the appearance of extracellular plaques (20). These observations precipitated a search for the soluble pathological Aβ culprit mediating cognitive decline, with emphasis shifting toward identifying the precursors to plaques. This led to the focus on soluble oligomeric Aβ species, aggregates up to ~150 kDa consisting of 2–30 Aβ peptides. As in AD Tg mice, cognitive decline in humans is not proportional to Aβ plaque load (21) but does correlate with soluble Aβ species (22). The latest data now indicate that soluble oligomeric species play a critical role in the pathogenicity of AD (23). Evidence supporting involvement of soluble Aβ oligomers in AD has come from human post-mortem brain tissue (24, 25); however, much of the evidence for the toxicity of oligomeric Aβ and its central part in AD has come directly from the use of Tg mouse models of AD. Using the Tg2576 mouse, Ashe and co-workers (26) recently identified a memory-impairing oligomeric Aβ species as a 56-kDa soluble protein assembly termed Aβ*56, which others have also confirmed (27). Intraneuronal Aβ has also gained experimental support in recent years (2). The accumulation of intracellular Aβ has been shown to precede extracellular deposition in both humans (28) and mice (29). In fact, it was found in Tg mice that intraneuronal Aβ strongly correlates with initial deficits in a hippocampus-based memory task (30), a result that supports a pathological basis of intraneuronal Aβ in mild cognitive impairment, which is often a harbinger for AD. Data from AD Tg mice also indicate that intraneuronal Aβ is more neurotoxic than extracellular Aβ (31).

Modeling Tau Pathology in Mice

Tau pathology is the other hallmark of AD. The Aβ cascade hypothesis predicts that tau hyperphosphorylation occurs as a downstream consequence of Aβ accumulation. APP-overexpressing Tg mice have provided evidence both for and against this. APP-overexpressing models do not develop NFTs, yet many do show detectable tau hyperphosphorylation (32). This could be because human Aβ accumulation does not induce NFT formation, although it is more likely that rodent tau has a different structure and sequence, rendering it more resistant to aggregate formation, and/or the life span of mice is not long enough to allow for hyperphosphorylation/aggregation, which implies that Aβ alone cannot induce tau pathology but requires an age-dependent cofactor or “second hit.” So although Aβ accumulation in APP-overexpressing mice does not lead to NFT formation, it should be remembered that these animals still develop robust cognitive decline and also undergo more subtle alterations in tau that resemble the precursors to NFTs in the human brain (most notably hyperphosphorylated tau). In fact, recent evidence has highlighted tau as being critical in mediating the cognitive decline brought about by accumulation of Aβ. It was shown that knocking out endogenous MAPT in APP-overexpressing mice prevented cognitive decline despite abundant Aβ accumulation (17). These data and others highlight a parallel to the Aβ peptide; it is the soluble species that mediates toxicity rather than the aggregated species. More so, it has been observed that Aβ oligomers can lead to tau pathology through activation of tau kinases such as GSK-3β (glycogen synthase kinase 3β) and through inhibition of the proteasome (33).

To model NFTs, it has been necessary to develop Tg mice that express further gene alterations in addition to mutant APP such as mutant human MAPT (29, 34) or removal of nitric-oxide synthase 2 (35). These multigenic AD Tg models do develop NFTs similar to those seen in human brain and have aided the explication of the relationship between Aβ and tau (33), with Aβ pathology seeming to precede the onset of tau pathology. In addition to providing evidence that Aβ accumulation occurs proximal to the onset of tau pathology, multigenic models of AD have also allowed us to determine how manipulation of Aβ affects tau and vice versa. Some of the strongest data supporting tau pathology as a downstream event of Aβ accumulation have come from the study of Tg mice. For example, in 3× Tg-AD mice, which contain human APP, PS1, and MAPT mutant transgenes, the appearance of intraneuronal Aβ precedes somatodendritic accumulation of tau (36). Furthermore, removal of intraneuronal Aβ via immunotherapy leads to
the removal of somatodendritic tau shortly afterward, provided tau is not aggregated (15). It was also found that Aβ oligomers inhibit proteasome function, which normally serves to degrade excess tau proteins, leading to tau accumulation (37). Such impairments in proteasome activity have been shown in human AD as well (38). Notably, reduction of tau pathology in these mice is necessary to ameliorate behavioral deficits (17, 27) and further supports the notion that Aβ accumulation impacts cognition via tau protein.

**Inflammation**

A further connection between Aβ aggregation and downstream pathologies, such as tau, exists in the inflammatory response present in AD. Inflammation in AD is not exactly modeled in mice, as there are differences between humans and AD Tg mice with respect to the nature and severity of the inflammation (39), yet AD Tg mice are still valuable for revealing which aspects of inflammation may be key for the development or elimination of downstream pathologies. Data from AD Tg mice indicate that inflammation, including activation of complement and various cytokines, occurs downstream from the aggregation of Aβ (7) and, more specifically, in association with fibrillar Aβ (40). Many of these inflammatory mediators that are up-regulated by Aβ can serve to increase tau pathology (33). Reactive oxygen species are also produced as a result of this inflammatory response (41), which is damaging to cell membranes and may further exacerbate the inflammatory response. In both humans and AD Tg mice, Aβ plaques are surrounded by activated microglia and astrocytes; thus, even as the activation of the inflammatory response in AD can lead to the detrimental effects discussed above, activated microglia act in a beneficial manner by attempting to phagocytose Aβ plaques (42). In support of the hypothesis that inflammation may have favorable effects in AD, acute inflammation, as brought about by treatment with lipopolysaccharide, has been shown to clear Aβ plaques (43) in AD Tg mice, whereas more chronic lipopolysaccharide treatment potentiates tau pathology (40). Aβ immunotherapy strategies have also proven useful in reducing plaque and, subsequently, tangle pathology as well as cognitive deficits in AD Tg mice (15, 44).

**Synaptic Defects**

AD Tg mice have not only allowed us to study the relationship of obvious AD pathologies such as amyloid accumulation and inflammation to one another, they have also allowed us to study aspects of AD that are inaccessible in humans and the effects of amyloid accumulation on such features. Whereas loss of synapses strongly correlates with cognitive deficits in humans (45), the relationship between synaptic deficits and progression of AD is more difficult to ascertain. For instance, LTP is a mechanism of synaptic plasticity implicated in learning and memory; however, LTP deficits in humans with AD can only be speculated upon. AD Tg mice have allowed us to determine that Aβ accumulation (and more specifically, intraneuronal Aβ) leads to impaired LTP in an age-dependent manner (46), supporting the notion that synaptic dysfunction may contribute to the cognitive deficits seen in AD. Recent evidence indicates that N-methyl-d-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, both instrumental to the development of LTP, are reduced in AD Tg brain in part due to their endocytosis following exposure to Aβ (47). The synaptic dysfunction in AD Tg mice precedes Aβ deposition (48), and in agreement with the damaging effects of oligomeric Aβ species on cognitive functioning discussed above, the synaptic dysfunction is especially correlated with soluble oligomeric Aβ species, with relevant data coming from AD Tg mice as well as studies involving the injection of oligomeric Aβ into rat brain (49). Remarkably, there is evidence for a negative feedback loop between Aβ production and synaptic transmission whereby increased neuronal activity leads to amplified β-secretase cleavage (and subsequent production of Aβ), whereas Aβ depresses synaptic transmission (50). One can speculate that this feedback loop is severely disrupted in the AD brain, leading to both synaptic dysfunction and unchecked Aβ production. AD Tg mice have thus been instrumental in allowing researchers to explore the physiological function as well as the pathological consequences of Aβ on synaptic function, which may be key to the memory impairments seen in the earlier stages of AD.

The effects of Aβ on synapse structure and function are of key interest given that one aspect of human AD not recapitulated in most AD Tg mice is abundant neuronal loss. The lack of cell loss in most AD Tg models has provided evidence that cognitive decline in AD may not be due solely to the loss of neurons. Although there is no significant cell loss in most AD Tg mouse models, virtually all AD Tg mice show cognitive deficits. In addition to the synaptic dysfunction discussed above, the memory deficits in AD, at least in the earlier stages, may be due to structural neuronal damage (such as loss of dendritic spines or synapses), which is seen in both humans (21) and AD Tg mice (51) or dysfunction at the level of the synapse. In fact, a recent study has given evidence that Aβ oligomers can induce loss of proteins involved in spine structuring and that this loss is well correlated with cognitive deficits (52). Tau pathology may also be important for the development of cognitive deficits because reduction in soluble tau (27) or in endogenous MAPT expression (17) is beneficial to the amelioration of cognitive impairments in AD Tg mice. Reduction in endogenous mouse tau has also been shown to decrease susceptibility to excitotoxicity (17). As Aβ has been shown to increase glutamatergic excitotoxicity (53), it may be that cognitive deficits in AD are due to excitotoxicity (as permitted by tau) in addition to (or in conjunction with) neuronal and/or synaptic loss and synaptic dysfunction. The fact that Aβ pathology occurs separately from both NFTs and neuronal cell loss in AD Tg mice has allowed researchers to investigate the distinct contributions made by each of these pathologies.

The involvement of Aβ-induced glutamatergic alterations and excitotoxicity compounds one specific concern with regard to Tg mouse models that has gained increasing attention in recent years: mouse genetic background strain. There are several characteristics that differ depending on mouse genetic background strain, and many of these may be highly relevant to the etiology, development, and consequence of Aβ pathology. Of specific relevance to AD research, mouse strain variation has been observed with respect to susceptibility to excitotoxins (54,
MINIREVIEW: Mouse Models of Alzheimer Disease

55) in that some strains are vulnerable to excitotoxic insult, whereas others (including the most commonly used AD Tg mouse background strain, C57/BL6) are not. Therefore, one reason underlying the lack of neuronal cell loss comparable with that seen in human brains in most AD Tg mice may be the inherent cellular resistance of current AD Tg models to excitotoxic damage from Aβ and downstream effectors. In addition, one research team has determined that background strain significantly modulates processing of APP as well as deposition of Aβ into plaques (56). These data indicate that generation of AD Tg mouse models should involve expression of human genes in mouse genetic background strains that allow for the most “human-like” Aβ aggregation and the most complete spectrum of downstream cascades from Aβ accumulation. It is not yet completely understood which mouse strain variations best mimic what is found in humans susceptible to AD, but it is interesting to speculate how some of the variability between mouse strains may be comparable with human populations such as those who develop SAD versus those who do not.

Future Directions

The etiology of the vast majority of AD cases still remains unknown. Existing models of AD solely mimic the rare familial forms of AD. One future goal will be to develop models of SAD. Future model development will seek to recapitulate AD-like forms of AD. One future goal will be to develop models of SAD. Unknown. Existing models of AD solely mimic the rare familial forms of AD. One future goal will be to develop models of SAD. Future model development will seek to recapitulate AD-like forms of AD. The etiology of the vast majority of AD cases still remains unknown. Existing models of AD solely mimic the rare familial forms of AD. One future goal will be to develop models of SAD. Future model development will seek to recapitulate AD-like forms of AD. Like virtually all tools, current AD Tg mice are limited in what is found in humans susceptible to AD, but it is interesting to speculate how some of the variability between mouse strains may be comparable with human populations such as those who develop SAD versus those who do not.

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MINIREVIEW: Mouse Models of Alzheimer Disease


75, 436–439