Modelling early responses to neurodegenerative mutations in mice

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Abstract

Considering the many differences between mice and humans, it is perhaps surprising how well mice model late-onset human neurodegenerative disease. Models of Alzheimer’s disease, frontotemporal dementia, Parkinson’s disease and Huntington’s disease show some striking similarities to the corresponding human pathologies in terms of axonal transport disruption, protein aggregation, synapse loss and some behavioural phenotypes. However, there are also major differences. To extrapolate from mouse models to human disease, we need to understand how these differences relate to intrinsic limitations of the mouse system and to the effects of transgene overexpression. In the present paper, we use examples from an amyloid-overexpression model and a mutant-tau-knockin model to illustrate what we learn from each type of approach and what the limitations are. Finally, we discuss the further contributions that knockin and similar approaches can make to understanding pathogenesis and how best to model disorders of aging in a short-lived mammal.

Introduction

The mouse is the most frequently used mammal for modelling late-onset human neurodegenerative disease. To interpret the data correctly, we need to consider the limitations of mouse models. Mouse lifespan is barely 2–3% of our own, even with high hygiene and nutritional standards and without predators. The mouse genome, although substantially similar to ours, has critical differences that influence the disorders we seek to model. For example, humans express a mixture of three- and four-repeat (3R and 4R) splice forms of MAPT (microtubule-associated protein tau), whereas adult mice express only 4R tau [1]. There are important cellular and anatomical differences too. For example, axonal transport impairment is important in many neurodegenerative diseases, but our axons have to transport cargoes over ten times as far as mouse axons, and human cortex has an area 1000-fold greater than in mice, with circuits for language and other processes that do not exist in mice.

Nevertheless, the mouse has many advantages that explain its frequent use. Relative to other mammals, it is low-cost, has a short generation time and is easy to manipulate genetically. There are impressive similarities between mouse models and corresponding human disorders, such as nuclear and neuritic inclusions in Huntington’s disease models, dystrophic neurites in amyloid models and such as nuclear and neuritic inclusions in Huntington’s disease show some striking similarities to the corresponding human pathologies in terms of axonal transport disruption, protein aggregation, synapse loss and some behavioural phenotypes. However, there are also major differences. To extrapolate from mouse models to human disease, we need to understand how these differences relate to intrinsic limitations of the mouse system and to the effects of transgene overexpression. In the present paper, we use examples from an amyloid-overexpression model and a mutant-tau-knockin model to illustrate what we learn from each type of approach and what the limitations are. Finally, we discuss the further contributions that knockin and similar approaches can make to understanding pathogenesis and how best to model disorders of aging in a short-lived mammal.

Key words: aging, Alzheimer’s disease, axonal transport, frontotemporal dementia, knockin mouse, microtubule-associated protein tau.

Abbreviations used: 3R, three-repeat; 4R, four-repeat; APP, amyloid precursor protein; FTDP-17, frontotemporal dementia with parkinsonism linked to tau mutations on chromosome 17; IAPP, microtubule-associated protein tau; MT, microtubule.

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models that naturally develop amyloid pathology with age and genetically modified mice that express mutant forms of human APP. Cerebral β-amyloidosis can occur in aged non-human primates and dogs [10,11]. In primates, Aβ deposition is associated with dystrophic neurites (neuritic plaques), whereas in dogs, the deposition occurs almost exclusively in diffuse deposits with no neuritic plaques. Therefore these animals could provide important insights into the mechanism of axon pathology caused by Aβ.

The advantage of using these models is that they have no overexpression, they live in a natural environment or share our environment and have a long lifespan. The pitfalls are the costs, restricted availability and ethical considerations.

Genetically modified mouse models of β-amyloidosis require the introduction of some combination of familial AD mutations into APP or presenilin 1 or both and often also overexpression of mutant human APP to drive the deposition of Aβ. Apart from these caveats, transgenic mice have been highly useful in showing that overproduction of Aβ leads to dystrophic axons and dendrites, and synapse loss, around the amyloid plaques [3,12,13]. The role of dystrophic neurites in AD pathogenesis remains unknown a century after they were described [14]. Reports of axon and dendrite interruption at amyloid plaques [15–17] and loss of dendritic spines [13,15,16] suggest mechanisms linking dystrophy to early synapse loss in patients [18].

In our recent study [3], we tested the hypothesis that dystrophic axons and dendrites are precursors of irreversible neuron damage by tracing them back to their cell bodies in double hemizygous TgCRND8/YFP-H mice. TgCRND8 mice inherit a highly aggressive amyloidopathy caused by expression of double-mutant APP (K670N/M671L plus V717F) [19], leading to early neurite dystrophy. YFP-H mice express YFP (yellow fluorescent protein) from a Thy1.2 promoter in a subset of neurons [20], which greatly facilitates tracing of axons over hundreds of microns in the CNS (central nervous system) and the detection of axonal dystrophy [21]. Thus, by tracing dystrophic axons back to the corresponding neurons, this enabled us to use axon and dendrite continuity, dendritic spines and branching, and nuclear size and location to indicate the viability of neurons whose axons were dystrophic.

Surprisingly, we found that almost all TgCRND8 axons retain continuity throughout the disease course, even at sites of extreme dystrophy. The corresponding cell bodies were morphologically normal, with unaltered nuclear size, and were likely to be metabolically active, as was evident by their support for both axons and dendrites (Figure 1). Although APP, synaptophysin and mitochondria all accumulated in sites of axon dystrophy, we surmise that axonal transport must continue to some extent across dystrophic regions to keep distal and proximal structures alive. Immunochemical and ultrastructural studies showed that dystrophic axons were tightly associated with disruption of presynaptic transmission machinery, suggesting local functional impairment.

Longitudinal imaging allowed us to view axonal, somatic and dendritic compartments of the individual neurons simultaneously. We found that many neurons encounter several plaques, either in different compartments or the same compartment, and suggest that a cumulative effect of many sub-threshold insults may eventually lead to axon...
degeneration and neuron death. This may be particularly the case in humans, since the damage may occur at a larger number of sites than in mice as axons are much longer, and an age-related decline in axonal transport may make axons particularly vulnerable in older individuals. Therefore we believe that our study models an early axonal response to amyloid pathology when axonal dysfunction is still localized, but axon degeneration and cell death have yet to occur. It also suggests that the therapeutic window for rescuing individual neurons could be considerably longer than suggested by the appearance of dystrophic axons, although it will be important to determine whether this is also the case in the brains of old humans.

A knockin model for FTDP-17T

The second distinguishing feature of AD are the intracellular neurofibrillary tangles composed of fibrillar aggregates of hyperphosphorylated tau which define a broad group of neurodegenerative diseases: the tauopathies. To date, myriad mouse models of tauopathy have been generated and many accurately model late-stage pathology (reviewed in [22,23]). However, most are transgenic lines that overexpress wild-type tau isoforms, or tau isoforms with disease-associated mutations. Whereas tau isoform ratios are altered in some tauopathies, the extent of tau isoform overexpression in most transgenic models is probably much greater than occurs in human disease. Even where there are relatively modest increases in total brain tau (2-fold or less), levels of exogenous tau expression in individual neurons can vary [20,24] (Figure 2). This raises the possibility that non-physiological tau expression in such models might itself influence pathogenesis in ways that differ from tau dysfunction in humans, even if the endpoints are similar. Furthermore, significant alterations in tau expression in specific neuronal populations due to the use of heterologous promoters could also underlie other changes in these mice, such as motor defects, that may be less relevant in human tauopathies.

We recently addressed these issues using a novel knockin approach [25]. We engineered mice with a mutation in the endogenous Mapt gene that is homologous with the common P301L MAPT mutation found in patients with FTDP-17T (frontotemporal dementia with parkinsonism linked to tau mutations on chromosome 17). Importantly, like FTDP-17T patients, these mice express P301L mutant murine tau from the targeted allele at physiological levels with the normal murine ratio of tau isoforms. However, unlike transgenic mice overexpressing human P301L mutant tau [26–30], neither heterozygous nor homozygous P301L-tau-knockin mice showed any signs of neurofibrillary pathology or any detrimental behavioural changes at any age. Critically, pre-tangle changes, such as tau hyperphosphorylation and/or tau aggregation, are also absent, despite the fact that they are often a feature of transgenic lines that overexpress wild-type human or mouse tau that are generally regarded as less pathogenic [24,31–35]. In fact, a wild-type tau transgenic line overexpressing the shortest tau isoform (0N3R) also shows some neurofibrillary tangle-like pathology [34,36]. This suggests that significant tau overexpression and/or profound changes in tau isoform ratios, rather than mutations, are required for late-stage tau pathology during the relatively short lifespan of a laboratory mouse.

However, this does not resolve whether tau overexpression simply accelerates pathogenesis in mice, or whether it causes early events that differ mechanistically from those in human tauopathies. Importantly, hyperphosphorylation of tau, which may be critical in the majority of tauopathies, and FTDP-17T mutations result both in a loss of normal tau function and a potential gain of toxicity [6,7]. In principle, either may contribute to pathogenesis, resulting in superficially similar late-stage tau pathology via different routes. Whereas transgenic tau overexpression models are more likely to model a toxic gain-of-function (or dominant-negative effects), the changes we have so far identified in P301L-tau-knockin mice, i.e. reduced tau phosphorylation and reduced MT (microtubule) association of tau, changes in axonal transport of mitochondria and hyperactivity, appear more consistent with a loss of normal tau function. Therefore, although there is no obvious tau pathology in P301L-tau-knockin mice, this study may be informative about critical early changes that are not revealed by toxic gain-of-function models.
Of course, many other factors could influence the ability to faithfully model the various human tauopathies in mice. These include inherent differences in the properties of human and murine tau and differences in the expression of 3R and 4R tau isoforms in the two species. However, the absence of any human tau, and more specifically human 3R tau isoforms, does not prevent mature tau tangle pathology from developing in some mouse models involving tau modulation [37,38]. Knockin models or transgenic mice with low level exogenous tau expression might benefit from some humanization since this could accelerate the pathogenic process, but it remains to be seen whether mouse models of tauopathy can be developed to precisely match specific human tauopathies, with overall levels of wild-type or mutant tau expression and 3R/4R tau isoform ratios that are equivalent to those in human patients.

Interestingly, in contrast with our P301L-tau-knockin model of FTDP-17T, physiological expression of some mutant proteins is sufficient to model pathology of other neurodegenerative diseases in mice. For example, chemically mutagenized mice homozygous for a D83G Sod1 mutation, which is pathogenic in human familial ALS (amyotrophic lateral sclerosis), show ALS-like motor neuron loss as early as 15 weeks [39]. Mutant huntingtin-knockin models have also been generated that develop Huntington’s disease-like pathology in their first year [40,41]. Thus not all human neurodegenerative disorders require overexpression for effective modelling in mice.

**Improving the accuracy of animal models**

To summarize the above, whereas overexpression of mutant transgens can model some aspects of pathology, most notably the cellular response to a toxic product such as Aβ, this approach has important limitations. We can be more confident that overexpression data represent endogenous disease processes if expression of a mutant allele at physiological levels in mice causes similar, even mild, pathology or cellular changes consistent with overexpression data. The different outcome in different diseases may be significant and we suggest that further knockin, spontaneous or chemically induced mutant mice are needed across a range of neurodegenerative diseases to increase our understanding. These studies may be slower, more expensive and involve more career risk than overexpression studies, but should not be neglected when they are scientifically crucial. Such models are also important for testing loss-of-function hypotheses, because the presence of a non-functional protein may be less likely to induce compensatory changes than complete absence of a protein. Thus we need to be wary of discounting loss-of-function hypotheses solely on the basis of a healthy knockout mouse.

Knockin, spontaneous or chemically induced mutations also produce the correct murine expression pattern and splice forms. The importance of this is emphasized by recent understanding of the importance of RNA-processing defects in ALS [42]. As BAC (bacterial artificial chromosome) transgens also retain the endogenous promoter and splicing, these have similar advantages in this respect.

In cases where knockin mice do not produce pathology, we may learn more from combining them. Combinatorial approaches have been widely used with overexpression models, as in triple-transgenic mice for example [43], but can now be refined to assess how pathogenic mutations interact at physiological expression levels. Similar studies in mice expressing multiple transgenes at low levels are already generating interesting new data and improving available models (see [44] in this issue of Biochemical Society Transactions). Knockin models should also be combined with environmental stresses to better mimic some of the pathogens, toxins and metabolic stress, etc., to which humans are regularly exposed [45].

In addition to controlling for the effects of overexpression using knockin mice, it is important to compare the effects of overexpression of mutant and wild-type proteins. Sometimes overexpression of wild-type and mutant proteins result in related, but mechanistically distinct, pathologies. For example, overexpression of wild-type tau causes increased tau phosphorylation and axonopathy, probably due to excessive MT binding, whereas reduced MT binding of P301L mutant tau in a matched transgenic line is the probable cause of the observed moribund tauopathy [30]. In some human neurodegenerative diseases, overexpression of the wild-type protein can itself be a cause. For example, extra copies of APP are likely to underlie the high prevalence of AD in Down’s syndrome, and α-synuclein is triplicated in some cases of Parkinson’s disease [46]. Thus mice overexpressing the corresponding wild-type proteins can be regarded as very close models of a subset of human patients. Indeed the corresponding transgenic models do show relevant pathologies [47,48], although the choice of promoter can still influence the phenotype [49].

Finally, we need to address the problem of extrapolating results from young mice to old people. Some of the more aggressive amyloid models show early lethality for reasons unrelated to AD pathology. This limits the study of how such transgens affect the aging brain. Following the successful use of inducible models to dissociate cognitive impairments and neuronal loss from tangle pathology in young mice [29] and to show reversibility of Huntington’s pathology in older mice [50], similar approaches can be used to repress aggressive transgens in young mice. In this way, disease could be induced in the aging mouse nervous system, which may be less able to withstand the effects and mimic human disease better.

**Funding**

This work was funded by the Medical Research Council [grant number G1000702] and Alzheimer’s Research UK [grant number ART/PG2009/2].
References


Received 6 May 2011
doi:10.1042/BST0390933