REVIEW

Tauopathy models and human neuropathology: similarities and differences

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Abstract Much of our current understanding of the pathogenic mechanisms in human neurodegenerative disorders has been derived from animal studies. As such, transgenic mouse models have significantly contributed to the development of novel pathogenic concepts underlying human tauopathies, a group of diseases comprising various forms of neurodegenerative disorders including Alzheimer's disease, corticobasal degeneration, argyrophilic grain disease, progressive supranuclear palsy, and Pick's disease as well as hereditary fronto-temporal dementia with parkinsonism linked to chromosome 17. Here, we will review in vivo models of human tauopathies with particular preference to transgenic mouse models. Strengths and limitations of these models in recapitulating the complex pathogenesis of tauopathies will be discussed.

Keywords Neurodegeneration · Tau · Amyloid-cascade · GSK3 · Phosphorylation · Microtubule

Introduction

Pathologic alterations in the microtubule-associated protein tau have been linked to the pathogenesis of a number of neurodegenerative disorders, including Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease (AgD), Pick's disease (PD), as well as a subset of hereditary frontotemporal dementias (FTDs), which are therefore also collectively classified as tauopathies [24, 49, 87]. In these diseases, the microtubule-associated protein tau accumulates, forming detergent-resistant intracellular inclusions known as neurofibrillary tangles (NFTs). In AD, the temporal and spatial distribution of tau pathology correlates well with the clinical disease severity [9]. However, whether it acts as the primary culprit or is indirectly involved in the pathogenic pathways culminating in neuronal cell loss, is still hotly contested. Although pathogenic tau mutations have been identified in FTD with parkinsonism linked to chromosome 17 (FTDP-17*T*) which is clear evidence of tau dysfunction playing a pivotal role in neurodegeneration, the mechanism of tau dysfunction leading to neuronal demise is only poorly understood.

In many ways, human tauopathies represent a heterogeneous group of diseases, with affected patients presenting with diverse clinico-pathological phenotypes ranging from prototypical aphasia to dementia syndromes. It is therefore of utmost importance to elucidate the mechanisms of tau accumulation and NFT formation in these diseases, and in particular to answer the question of whether all tauopathies develop through a unifying common pathogenic pathway. On the molecular level, the phenotypic diversity of tauopathies is attributable to different types of tau gene mutations as well as additional modifying genetic and epigenetic factors. Whereas on the cellular level, not only neuronal but also glial cell elements can be affected by tau-related pathology in locoregionally different and disease- (and sometimes also stage-) specific patterns, heterogeneity on the biochemical level is reflected by the expression of different tau isoforms. These isoforms arise through alternative splicing, yielding variants with differential binding to microtubules according to the presence of either three (3R isoforms) or four microtubule-binding repeat domains (4R isoforms). Homologous repeat sequences are also present in

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the microtubule-associated proteins MAP2 and MAP4. As a major microtubule-associated protein, mammalian tau is subject to extensive regulation on the transcriptional level and to post-translational modification, in particular to phosphorylation, resulting in complex biochemical patterns [51]. Through binding to microtubules, tau is involved in the initiation and stabilization of these structures, which, in neurons, are critical for axonal transport and proper synaptic function [84]. Of particular note, overexpression of tau in neuronal cell lines as well as in primary neurons leads to disturbed kinesin-dependent trafficking of organelles along microtubule tracts [21, 86]. In particular, mitochondria fail to be transported to peripheral cell compartments, which may represent an important pathogenic mechanism for neurodegeneration [79], contributing to the loss of synapses as an early event in AD pathogenesis [14, 83].

In the adult human brain, six tau isoforms are expressed that are generated through alternative splicing [12, 23, 49]. These isoforms differ by containing either three or four microtubule-binding domains within their C-termini (3R and 4R isoforms, respectively) with additional variability arising from the existence of none, one or two N-terminal inserts (0N, 1N, 2N isoforms). The 4R isoforms bind to microtubules more avidly than the 3R variants. Isoform expression patterns are developmentally regulated with the 3R variants being expressed during early life phases ("fetal tau") and it has therefore been hypothesized that expression of 3R tau might be associated with the higher degree of plasticity that is required during neuronal development [26]. Although in embryonic hippocampal slice cultures of tau-null mice an inhibition of neuronal maturation can be demonstrated [19], tau-deficient mice appear physiologically normal, are able to reproduce and do not develop obvious neurological phenotypes, presumably because other microtubule-associated proteins (such as MAP1A and 1B) can compensate for tau loss [31].

For quite some time, interest in tau as an important pathogenic factor in AD-related neurodegeneration has lagged behind the research efforts devoted to the amyloid beta peptide ($A\beta$), partly because tau pathology was commonly accepted to occur downstream of $A\beta$ according to the beta amyloid cascade hypothesis [32]. However, in various other tauopathies, prominent tau pathology develops in the absence of extracellular $A\beta$ deposits. In addition, the identification of tau mutations underlying the pathogenesis of FTDP-17T [39, 68, 75], a neurodegenerative FTD entity which follows an autosomal-dominant mode of inheritance, has sparked intense research efforts aimed at the generation of animal models not only to decipher the role of tau in neurodegeneration but also at deriving novel therapeutic approaches for human tauopathies.

Remarkably, several crucial features of human tauopathies have been recapitulated in relatively simple organisms, such as *Caenorhabditis elegans* and *Drosophila melanogaster*. However, in these models many pathogenic aspects are overly simplified and therefore considerably limit the interpretation of findings in terms of drawing parallels to the pathogenic pathways at play in human tauopathies. Consequently, various tau transgenic (tg) mouse lines have been generated to closer model aspects of the very complex, multi-facet pathogenesis of human tauopathies. After briefly summarizing selected findings in *C. elegans* and *D. melanogaster*, we will focus this review on the various tg mouse models that have been generated to experimentally recapitulate important histopathological, and sometimes also behavioral features of tauopathies.

Modeling tauopathies in *C. elegans*

Caenorhabditis elegans represents an easy to genetically manipulate model for the experimental characterization of normal gene function as well as for exploring pathogenic protein dysfunction. To model tauopathy disorders, several transgenic worm models have been generated [10, 46, 61]. Neuronal expression of normal and FTDP-17T associated mutant human tau proteins (P301L, V337M) [46] caused progressive uncoordinated locomotion, indicative of a nervous system defect, which culminated on day 9 with worms becoming nearly immobile. Moreover, expression of wildtype (wt) human tau was associated with a decreased life span [46]. Interestingly, the transgenic tau protein expressed in *C. elegans* was found to be phosphorylated at analogous residues as it occurs in human tauopathies [46], an observation recently confirmed independently [10]. Of note, whereas no neuronal degeneration was reported by the latter group, transgenic human tau was demonstrated to become highly phosphorylated and to assume a PHF-like conformation [10]. Significantly, FTDP-17T mutant tau lines exhibit a more severe phenotype marked by the production of more detergent-insoluble tau and by a more severe axonal degeneration in comparison to wt-tau expressing lines [46]. In particular, in the line expressing the FTDP-17T-associated V337M tau mutation, the high level of insoluble tau was paralleled by extensive axonal degeneration [46]. Nerve cell degeneration, accompanied by tau-accumulation in cell bodies and neuronal processes, was recently also reported for another transgenic worm model where the P301L and R406W mutations were expressed in mechanosensory neurons [61]. Thus, the induced tau pathology in worms resembles the situation in human tauopathies in so far as there is progressive accumulation of hyperphosphorylated insoluble tau as well as neurodegeneration with one of the main differences being the absence of overt tau tangle formation in *C. elegans*. Nevertheless,

in summary, these experiments provided evidence for at least a contributory (if not primary causal) role of tau in neurodegenerative processes and thus points to a high degree of evolutionary conservation in tau-associated pathogenesis.

Lessons from Drosophila

Bridging the simplicity of *C. elegans* and the complexity of vertebrate models, *D. melanogaster* has become an invaluable tool for the study of neurodegenerative diseases [6]. At least 50% of *Drosophila* genes have human homologs and more than 70% of human disease genes have *Drosophila* homologs, making the (rather primitive) fly a relevant organism to model selected aspects of human diseases, including neurodegenerative disorders. *Drosophila* flies possess one single tau gene, which is expressed in many types of neurons, where in analogy to mammalian neuronal cells, tau protein can also accumulate within axonal processes [33]. Wittmann et al. [95] have generated transgenic *D. melanogaster* flies as a genetic model of tau-associated neurodegenerative disorders by expressing wt and mutant (R406W, V337M) forms of human tau which recapitulated key features of human tauopathies. Despite only moderate levels of transgene expression, transgenic flies exhibited adult onset progressive neurodegeneration with a significantly reduced life span, especially in the R406W tg fly lines. With relative anatomic selectivity abnormal tau protein accumulated, but NFT formation was not observed. In summary, these results indicated that tau-induced neurodegeneration in flies does occur in the absence of large tau filamentous aggregates [95]. Moreover, overexpression of the human tau homolog in *Drosophila* neurons induces apoptosis, which is not accompanied by the formation of intracellular NFTs [6, 22, 43]. This finding, which, *prima vista*, would argue against a significant cytotoxic role of tau-rich NFTs, could reflect a mode of neurotoxicity which depends on protein alterations occurring prior to the assembly of fibrillar aggregates such as on smaller proteolytic tau fragments, which—at least in the human system—were recently postulated to be pathogenically important by promoting aggregation through nucleation effects [50, 67, 93]. However, when wt human tau (4R isoform) was coexpressed with the fly homolog of glycogen synthase kinase-3beta (one of the known tau-phosphorylating kinases) in another *Drosophila* model, NFTs were observed [43]. Thus, *Drosophila* tau models may faithfully replicate several important aspects of human tauopathies, including tau hyperphosphorylation, accumulation, and fibril formation as well as neuronal degeneration.

Mouse models

A considerable number of mouse tauopathy models have been generated that not only recapitulated key histopathological hallmarks of tauopathies but, to a variable degree, also reproduced important behavioral aspects of the respective human disorders. Tauopathies are morphologically heterogeneous disorders, with neuronal and/or glial cell components affected in rather disease-specific and sometimes stereotypic spatiotemporal patterns [24, 49, 87]. On the molecular level, it should be noted, that in contrast to the adult human brain only 4R variants of the murine tau homolog are endogenously expressed in the adult mouse brain. Furthermore, major AD-related phosphorylation sites of human tau are conserved in the murine protein, including those phosphorylated by the major tau kinases glycogen synthase-kinase 3β (GSK-3β) and cyclin-dependent kinase 5 (CDK5), both of which also serve crucial roles during neurodevelopment [16, 17, 44]. Of note, with regard to AD pathogenesis, β-amyloid has been shown to trigger tau phosphorylation by activating GSK-3β and CDK5 [44, 48]. It therefore seems that the mouse lends itself as a model organism to recapitulate essential aspects of tauopathies. However, as will be discussed below, extreme care needs to be exercised in the interpretation of histopathological findings and the results of behavioral tests. Depending on the promoter system chosen to drive transgene expression, the phenotypes of tg mouse models often differ strikingly even if the very same transgene is expressed. Whereas, the murine thy-1 promoter favors transgene expression especially in brain stem and spinal cord (which consequently often results in an axonopathy-related pathology through the preferential affection of the long fiber tracts), others such as the α-calcium-calmodulin-dependent kinase-II (CaMK-II) promoter target expression predominantly to the forebrain giving rise to behavioral phenotypes. In addition, gene dosage effects and altered ratios of expressed tau isoforms (i.e., 4R versus 3R variants) have to be taken into account. Furthermore, through the use of additional promoters, cell-type specific transgene expression (neuronal versus glial expression) can be achieved. Moreover, different promoters yield variable transgene expression levels, which is extremely relevant as pathological protein aggregation including the assembly of fibrillar protein deposits is concentration-dependent. Last but not least, as will be discussed in more detail below, phenotypes of tg mice show striking variance depending on whether transgene expression occurs in the murine endogenous or in a knockout background. Thus, currently existing tg mouse models can only recapitulate selected aspects of human tauopathies. With regard to the multiple molecular pathways that have so far been implicated with the rather complex pathogenesis of these diseases, it currently seems more than questionable

whether the generation of mouse models which faithfully mirror the full human disease phenotypes will ever be achieved. Rather, a more realistic view seems that by carefully putting together the single pieces of information that are provided by each of the mouse models, a picture will emerge in the hopefully not too distant future that permits understanding the complex pathogenic pathways in tauopathies well enough to devise rationale, effective and possibly individualized therapeutic approaches to combat these devastating diseases.

Models with tg expression of human wt tau isoforms (Table 1)

Modeling neuronal pathology

The initial mouse models for human tauopathies allowed only specific human tau isoforms to be studied within the background of endogenous wt murine tau. The first tg mouse models were generated more than a decade ago, when pathogenic tau mutations had not yet been identified, by expressing the longest human brain tau isoform (4R/2N) under the control of the human thy-1 promoter [29]. The tg human tau protein was found to be expressed in neuronal cell bodies, axons and dendrites. Interestingly, the tg tau protein, expressed at rather low levels, was phosphorylated at the same sites that are hyperphosphorylated in the paired helical filaments in human AD brains. In the absence of NFTs this observed pathology [29] was therefore interpreted as a pre-tangle phenotype, to be considered as intracellular "precursor" lesion preceding the full-blown tau NFT pathology associated with tauopathies [3, 8]. In a similar mouse model where the human 4R/2N isoform was expressed under the control of the murine thy1 (thy-1.2, respectively) promoter, prominent somatodendritic tau expression and hyperphosphorylation was demonstrated [69, 77] (Fig. 1a, b). These mice exhibited severe axonopathy with signs of Wallerian degeneration reflected by axonal breakdown and myelin sheath disintegration. Moreover, severe motor symptoms with neurogenic hindleg muscle atrophy, as histologically verified by the presence of grouped small angular muscle fibers, developed in these animals [69, 77].

In further models, alternative promoters such as the one for mouse prion protein (MoPrP) were used to control transgene expression which resulted in even more pronounced phenotypes. Two tg models, in which the shortest human brain isoform of tau (3R/0N) was expressed, were created [11, 41]. Brion et al. [11], using a mouse model with rather low 3R tau transgene expression driven by the murine, house-keeping type HMG-CoA-reductase promoter, did not find NFTs in mice younger than 19 months of age, but the tg protein was demonstrated to be phosphorylated

at sites known from tau proteins of paired helical filaments. In contrast, using the MoPrP promoter to drive transgene expression, insoluble, hyperphosphorylated tau progressively accumulated and NFT-like argyrophilic intraneuronal inclusions consisting of tau-immunoreactive filaments were observed in these mice as early as at 6 months of age [41]. Due to high-transgene expression in spinal cord motor neurons, variable axonopathy with formation of tau-immunoreactive spheroids and histomorphological correlates of axonal degeneration was found. Of note, the tau inclusions, although stainable by the Bodian silver method, were not detectable by the Gallyas procedure, which detects most NFTs in human tauopathies [90]. Nevertheless, although formation of *bona fide* NFTs was not observed (unless the mice reached a very old age [42]), these were the first tg mice to recapitulate key features of human tauopathies. The follow-up analysis of these mice at 12–24 months of age [42] revealed that the fibrillary tau lesions had now converted into AD-like tangle structures detectable by the Gallyas silver impregnation method. The particular relevance of this model was that, for the first time, an age-dependent maturation of tau inclusions culminating in the assembly of insoluble, filamentous, Gallyas-positive NFTs was experimentally recapitulated.

As pointed out earlier, the interpretation of these mouse models, while being valuable first steps to explore the role of tau in neurodegeneration, was restricted by the fact that only one specific isoform of human tau was transgenically expressed. To avoid this caveat, alternative novel mouse models were created. Duff et al. [20] generated mice that overexpressed a human tau transgene containing not only the coding regions but also intronic as well as regulatory sequences. Thus, in these mice, all six human tau isoforms were expressed at the mRNA and protein level in the CNS but when compared to human brain the ratio of 3R:4R human tau isoforms differed as relatively more 3R tau was present in these mouse brains. However, due to the presence of 4R endogenous murine tau there was no imbalance of the total 3R:4R ratio. Tau-positive axonal swellings developed in the spinal cords of the mice and correlated with a hind-limb abnormality, but no other neuropathological abnormalities, in particular no tangle formation, were seen [20]. In an even more sophisticated approach, Andorfer et al. [2] generated a tg mouse model that produces all six human tau isoforms in the absence of endogenous mouse (4R) tau, shifting the isoform ratio toward the 3R variants. Notably, these mice developed tau filaments by the age of 9 months, and pathological tau accumulated in cell bodies and neuronal dendrites in a spatiotemporal-relevant pattern. Resembling AD pathology, tau aggregation predominantly occurred in the neocortical neurons and pyramidal hippocampal cells, whereas brain stem and spinal cord were almost devoid of tau lesions. There was no

Table 1 Transgenic mouse strains modeling tauopathy through human wt tau expression

Transgene	Promoter	Histopathology	Phenotype	Remarks	References
4R/2N	Human Thy-1	Phosphorylated tau; somatodendritic tau distribution		Relatively low transgene expression, pre-tangle tau pathology, no NFTs	[29]
4R/2N	mThy-1 (mThy-1.2 in [69])	Somatodendritic staining for hyperphosphorylated tau, early axonopathy with degeneration in brain and spinal cord; astrogliosis	Progressive motor deficits; unable to spread hindlimbs when lifted by the tail; neurogenic muscle atrophy, normal life span	Pre-tangle tau pathology, no NFT formation	[69, 77, 85]
4R/2N × GSK3β	mThy-1	Axonopathy rescued			[78]
4R/2N	MoPrP	Weak somatodendritic tau staining		No NFT formation	[98]
3R/0N	mHMG-CoA reductase	tg Tau expressed in neurons (cell bodies and dendrites) and few astrocytes		Pre-tangle tau pathology, no NFT formation	[11]
All 3R isoforms of human tau	mtubulin Tα1	Accumulation of tau in astrocytes and oligodendrocytes with subsequent cell death; disruption of myelin sheaths; no neuronal tau lesions	Motor deficits (weakness in hind- and forelimbs) associated with age-dependent accumulation of insoluble hyperphosphorylated human tau	Glial tau lesions similar to astrocytic plaques and oligodendroglial coiled bodies in old mice	[34]
3R/0N	MoPrP	Rare NFTs in aged mice (24 months); axonal degeneration	Progressive motor deficits		[41, 42]
All six isoforms of human tau, endogenous murine tau background	Human tau	Tau-immunoreactive axonal swellings in spinal cord	Hind-limb abnormality	High 3R:4R ratio, no NFTs	[20]
Same as above, but on murine tau-null background	Human tau	Hyperphosphorylated tau accumulating as PHF	Mice fertile and viable	Filamentous tau pathology in spatiotemporally relevant distribution	[2]
4R/2N on murine tau-null background	mThy-1	No obvious pathology; significant increases in hippocampal volume and neuronal number	Minor motor phenotype late in life, normal mean life span; improved cognitive function	4R Tau important for hippocampal development by controlling neuronal precursor proliferation and differentiation	[74, 85]

Fig. 1 **a** Abundant tau stained (phosphorylation-dependent anti-tau antibody AT8) pre-tangle neurons are observed in sector CA1 of a transgenic mouse strain expressing the longest human four-repeat tau isoform [64]. **b** Notably this pre-tangle pathology is not stained by the Gallyas silver technique. **c** AT8 stained neurofibrillary tangles (*NFT*) in the brainstem region of a transgenic mouse strain expressing the human P301S mutation [1]. **d** In contrast to the pre-tangle pathology obtained in mouse strains expressing human wild-type tau isoforms NFT in P301S mice are strongly stained with Gallyas. *Scale bar* **a**–**d**: 100 μm
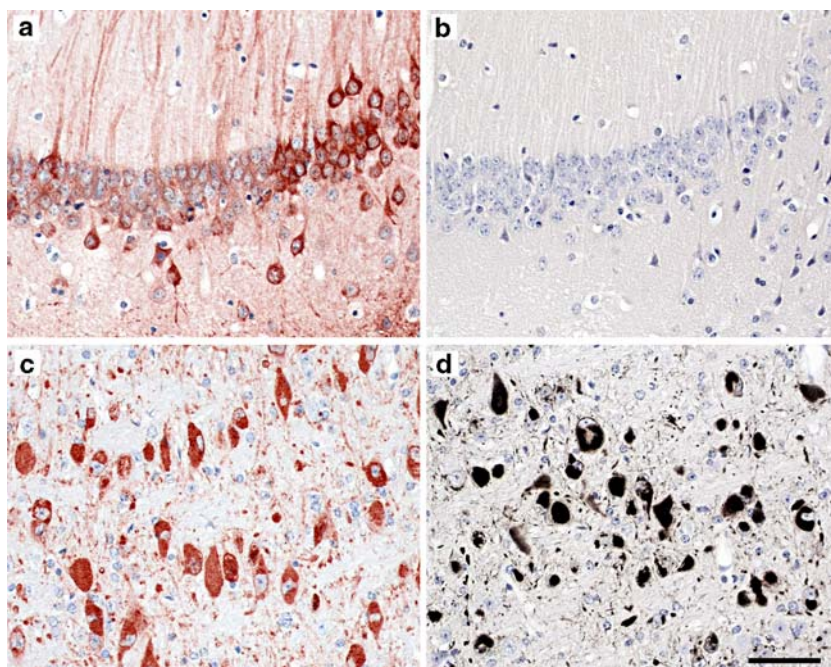
evidence of significant motor or behavioral abnormalities. Subsequently, Van Leuven and co-workers [85] established a mouse model where the human tau-4R/2N isoform was expressed in a tau-null background backcrossed into FVB/N. These mice survive normally and display only a minor motor phenotype, which occurs late in life in the absence of tau aggregates [85]. Of note, also in contrast to 4R/2N tg mouse models with the endogenous mouse tau background [69, 77] no obvious pathology, in particular no axonopathy was observed [85]. Instead, in a very similar model of mice expressing the 4R/2N isoform in a murine tau-k.o. C57/BL6 background analysis revealed significant increases in hippocampal volume and neuron numbers with improved hippocampal memory retention [74]. It was concluded from this study that 4R-tau serves an important function during hippocampal development by controlling neuronal precursor proliferation and differentiation [74]. Interpreting the results of these tg models, the ensuing question of whether endogenous murine tau is inhibitory to tau filament formation, remains to be answered unambiguously. The large excess of wt human tau in most of these models suffices to induce mild "pre-tangle" pathology, marked by an abnormal non-filamentous accumulation of phosphorylated tau in cell bodies and dendrites with an associated axonopathy and variable motor symptoms. However, even with the use of strong promoters, high-level expression of wt tau is—with two exceptions [2, 42]—not sufficient to cause the formation of *bona fide* NFTs as well as significant neuronal losses. Remarkably, especially in light of the amyloid cascade hypothesis, this also holds true for mice that coexpress amyloid precursor protein (APP) harboring the Swedish and London mutations (APP_{751SL}) and the presenilin-1 M146L mutant ($PS1_{M146L}$) combined with the human 3R/0N wt tau transgene under control of a modified HMG-CoA reductase promoter [7]. Only when the human 3R isoform is expressed under control of the MoPrP promoter, few Gallyas-positive NFT-like structures are observed in mice aged 24 months [42]. Moreover, the Andorfer et al. study [2] recapitulated for the first time tau hyperphosphorylation with formation of fibrillary tau aggregates by transgenically expressing all six human tau isoforms in a murine tau-k.o. background.

Modeling glial pathology

While the tg mouse models discussed above strongly focused on studying tau in CNS neurons, the filamentous glial tau pathology characteristic of CBD, PSP, AgD, and most FTDP-17*T* cases had been neglected so far. Götz et al. [30] and Higuchi et al. [34] were the first to report on tg mice that modeled tau-associated neurodegeneration of glial cells. When all three isoforms of human 3Rtau were expressed under the control of the mouse Tα1 α-tubulin promoter, filamentous, Gallyas-positive aggregates reminiscent of astrocytic plaques were observed which—although not specific—are considered rather typical lesions in CBD [13]. These lesions developed in older mice and no formation of tau aggregates was detected in neurons [34]. Furthermore, recapitulating typical findings in PSP, CBD, and AgD, Gallyas-positive, tau-immunoreactive oligodendroglial coiled body-like structures were found which correlated with an age-dependent accumulation of filamentous

tau in degenerating oligodendroglial cells [34]. Thus, the formation of tau-positive glial lesions as they occur in certain human tauopathies can be modeled in transgenic mice. As will be discussed in the following section, FTDP-17*T*-associated tau mutants were also expressed using alternative glia-targeting promoters to model human glial tau pathology [30, 36].

Models with tg expression of human mutant tau isoforms (Table 2)

Modeling neuronal pathology

Soon after the identification in 1998 of pathogenic tau mutations in a subset of FTDP-17 families (reviewed in [92]), various groups were successful in creating tg mouse models in which NFT formation was recapitulated in vivo. Both, neuronal [1, 27, 53, 80, 82] and glial tau filamentous pathologies [30, 36] were demonstrated. Tau gene mutations that underlie the pathogenesis of certain hereditary tauopathies such as FTDP-17*T* either occur near splice sites, changing ratios within the expressed tau isoform repertoire, or occur as missense mutations, both of which likely affect the binding affinity of tau toward microtubules. In the first reported mouse model which successfully demonstrated the formation of NFTs, the human P301L mutation, which results in pronounced adverse effects on microtubule-assembly promoting activity [4], was expressed under the control of the MoPrP promoter [53] leading in homozygous mice to mutant human tau expression at a twofold level in comparison to endogenous murine levels. In homozygous mice, motor and behavioral deficits related to NFT formation started to develop at 4.5 months with NFTs and Pick body-like neuronal lesions occurring in amygdala, mid-brain, brain stem and spinal cord, and with pre-tangle tau pathology in the neocortex, hippocampus, and basal ganglia. In addition to a significant reduction of spinal cord motor neurons that almost reached 50%, a peripheral neuropathy accompanied by neurogenic muscle atrophy was observed. Biochemically, the presence of Gallyas-positive NFTs containing hyperphosphorylated tau was demonstrated. One of the significant advances achieved through this model was that, for the first time, a link between neurofibrillary pathology and neuronal loss was established. Remarkably, a relatively low transgene expression level was sufficient to trigger fibrillary tau aggregates. In contrast, previous mouse models with higher expression levels of the human wt tau transgene failed to cause NFT formation, which likely reflects the pathogenicity of P301L as the most common tau gene mutation associated with FTDP-17*T* (reviewed in [92]).

Expressing the same P301L transgene, however under the control of the murine thy-1.2 promoter, Götz et al. [27] similarly observed hyperphosphorylation of accumulated tau which appeared redistributed from its normal axonal localization to somatodendritic compartments. In addition, as demonstrated by electron microscopy, short filament formation occurred in the cortex, brain stem and spinal cord [27]. Likewise, using a very similar model controlled by the murine thy-1 promoter, wt human 4R/2N tau was expressed in one mouse strain comparatively to the P301L mutation [85]. Whereas in the wt tau mice motor symptoms were evident already at 6–8 weeks, no tau aggregates formed and the animals had a normal life span. In contrast, in the P301L transgene-expressing mice, progressive fibrillary tau aggregation started to develop at around 6 months of age and, despite only minor motor problems, animals died prior to the age of 13 months [85]. Tangle-like tau pathology was also observed in mice that expressed the P301L mutation in combination with a tet-off regulated CaMK-II promoter system [70, 72]. As expected, high-level P301L expressing mice developed pre-tangles as early as at 2.5 months of age, with rapid progression of tau pathology culminating both in NFT formation by around 4 months of age and marked cognitive impairment. NFTs were first observed in the neocortex and, with increasing age, progressed to the hippocampus and the limbic system [70, 72]. Remarkably, when transgenic P301L expression was suppressed by administration of doxycycline, memory function was significantly improved, but NFTs continued to accumulate, and a region-specific dissociation between neuronal loss and the occurrence of NFTs was verified [76]. This may also be related to a postulated dissociation between the mechanisms causing memory impairment and those leading to tau fibrillation. It seems that in this model NFTs per se are not the primary cause of the observed cognitive deficits [72] which may rather be reflections of a reversible neuronal dysfunction.

Interestingly, proteomic analyses of P301L tg mice revealed a mitochondrial dysfunction related to modification in the expression patterns of mitochondrial respiratory chain complex components, antioxidant enzymes implicated in detoxification of reactive oxygen species (ROS) and of synapse-associated proteins [18]. In the context of tauopathies, mitochondrial dysfunction has also independently been corroborated in vivo by several groups. For instance, chronic rotenone-mediated complex I inhibition is sufficient to trigger a cerebral tauopathy with tau accumulations occurring in neurons, astrocytes, and oligodendrocytes [38].

In mice expressing the human P301S tau mutant associated with early onset FTDP-17*T*, two groups demonstrated neurodegeneration leading to nerve cell loss. In these models starting at 5–6 months, filamentous hyperphosphorylated tau pathology develops in brain stem and spinal cord with a significant reduction of spinal cord motor neurons in

Table 2 Transgenic mouse strains modeling tauopathy through human mutant tau expression

Transgene	Promoter	Histopathology	Phenotype	Remarks	References
P301L (4R/0N)	MoPrP	Age- and dose-dependent NFT development, starting at 4.5 months; neuronal loss, neurogenic muscle atrophy	Severe motor and behavioral deficits, premature death	NFT pathology linked to neuronal loss	[53, 55]
P301L (4R/2N)	mThy-1.2	NFT-like, sarkosyl-insoluble, short filament structures with hyperphosphorylated tau at 8 months; gliosis, TUNEL-positive neurons			[27]
P301L (4R/2N)	mThy-1	Widespread NFTs at 6 months; no axonopathy	Late minor motor phenotype, muscle and tissue wasting, death prior to age of 13 months	Tau hyperphosphorylation, progressive aggregation	[85]
P301L (4R/0N) (tet-regulatable)	CaMK-II	High transgene expression levels in forebrain with progressive, age-related NFTs and neuronal loss; pre-tangles starting at 2.5 months	Early cognitive impairment	Upon transgene suppression, significant improvement of memory function, despite ongoing NFT accumulation	[70, 72]
P301L (4R/1N)	2',3'-CNP	Impaired axonal transport before axonal degeneration; disruption of myelin and axons preceding filamentous oligodendroglial tau inclusions	Weight loss and progressive motor weakness, presumably as a consequence of neurogenic muscle atrophy	Oligodendroglial inclusions Gallyas-positive	[36]
P301S (4R/0N)	mThy-1.2	Abundant filamentous tau in brain stem and spinal cord; significant reduction of spinal cord motor neurons with neurogenic muscle atrophy	Severe paraparesis at 5–6 months	Soluble tau demonstrated to be phosphorylated prior to filament assembly; microglia adjacent to tau-positive neurons	[1, 5]
P301S (4R/1N)	MoPrP	Early synaptic pathology with dysfunction at 6 months of age, before neuronal loss and NFT formation; hippocampal and cortical atrophy by 9–12 months of age	Limb weakness, progressing to paralysis; median survival 9 months	Early prominent microglia activation, prior to tangle formation; immuno-suppression attenuates tau pathology	[97]
G272V (4R/2N) (tet-regulatable)	MoPrP	Transgene expression in neurons and oligodendrocytes	No obvious neurological deficits	Tau filament formation	[30]
G272/P301S double mutant (4R/1N)	mThy-1.2	NFTs; pathology starting at 6 months of age in hippocampus (CA1) and neocortex; ghost tangles at 12 months	Decreased synaptic transmission; behavioral abnormalities (anxiety, cognition, memory); no motor deficits		[73]
G272V/P301L/R406W triple mutant (4R/2N)	mThy-1	High concentration of hyperphosphorylated somatodendritic tau in cortex and hippocampus; sarcosyl-insoluble filament formation		Lysosomal abnormalities observed in forebrain	[54]
V337M (4R/2N)	PDGF-β	Degenerating hippocampal neurons, immunoreactive for PHF-associated tau; neuronal cell death	Decreased hippocampal neural activity, cognitive deficits (elevated plus maze)		[81]

Table 2 continued

Transgene	Promoter	Histopathology	Phenotype	Remarks	References
R406W (4R/2N)	MoPrP	Tau lesions with advancing age in neuronal perikarya	Hindleg weakness in older mice		[98]
R406W (4R/2N)	CaMK-II	Congophilic hyperphosphorylated tau in forebrain at 18 months of age; insoluble tau filaments in aged mice only	Impaired associative memory (fear conditioning), no obvious sensorimotor deficit		[82]
R406W (4R/2N)	Syrian hamster PrP	Phosphorylated, sarkosyl-insoluble, Gallyas-positive tau starting to accumulate in hippocampus and amygdala at 6 months of age	In <20% of tg mice, motor symptoms, and progressive acquired memory loss between 10 and 12 months	In more than 80% of tg mice, no significant behavioral or neuropatho-logical phenotype; reason for variable expression/penetrance unclear	[40]
APP_{sw} × P301L (4R/0N)	MoPrP	NFT pathology in limbic system and olfactory cortex at 6–9 months of age (enhanced as compared to P301L mice); starts at 3 months of age in spinal cord and pons	Motor symptoms similar to P301L mice [51], with progressive hindlimb weakness, hunched posture, eye irritations, etc.	APP (and/or $A\beta$) promote NFT formation	[52]
APP_{sw} × $PS1_{M146V}$ × P301L	mThy-1.2	Progressive development of plaque and tangle pathology	Synaptic dysfunction prior to onset of $A\beta$ and tau pathology		[66]
hAPP × tau +/− hAPP × tau −/−		No changes in hAPP or $A\beta$ (soluble or aggregated) levels observed	Cognitive deficits of APP mice prevented by decreased levels of endogenous tau expression	Study demonstrates link between neuronal overexcitation and cognitive impairment	[71]

homozygous mice in conjunction with severe paraparesis [1, 5, 97] (Fig. 1c, d). Remarkably, Bellucci et al. [5] also reported on microglia activation—recently implicated in the clearance of Aβ deposits (for review see [96])—adjacent to tau-positive neurons.

In an alternative model of FTDP-17*T*, the human V337M mutation that is weaker in comparison to the P301L mutation with regard to affecting microtubule-assembly was expressed under the control of the PDGF-β promoter. This resulted in the formation of tau-containing NFTs in the hippocampus and in various behavioral abnormalities [81]. Similarly, when the FTDP-17*T*—associated R406W mutation was expressed using the CaMK-II promoter, congophilic hyperphosphorylated tau inclusions formed in the forebrain starting at 18 months of age, with aged mice exhibiting an impaired associative memory but no significant sensorimotor deficits. Sarcosyl-insoluble tau was recovered from old tg mice only [82]. Expression of the same mutant, but under control of the Syrian hamster prion protein promoter, led to the development of filamentous tau aggregations in the hippocampus and amygdala with subsequent spreading of tau pathology to neocortical and subcortical regions. Accumulated, sarcosyl-insoluble tau was found to be phosphorylated, ubiquitinated, and stainable with the Gallyas silver impregnation method [40]. Again, in analogy to the P301S mouse model reported by Bellucci et al. [5], a pronounced activation of microglia was prominent. Behavioral tests of the R406W tg mice revealed motor symptoms as well as progressive loss of acquired memory at the age of 10–12 months [40]. In a third tg human tau R406W mouse strain, with expression driven by the MoPrP promoter, Zhang et al. [98] similarly observed filamentous tau lesions with advancing age, which occurred in neuronal perikarya of the cerebral cortex, hippocampus, cerebellum, and spinal cord. Interestingly, when compared to otherwise identical tg mice expressing human wt tau, reduced tau levels were found in CNS axons and linked to retarded axonal transport, presumably resulting from an accumulation of insoluble R406W but not human wt tau protein [98]. In yet another model, where G272V and P301S mutations were coexpressed in a thy-1.2-regulated manner, hyperphosphorylation and Gallyas-positive NFT-like pathology were observed. These mice developed defective hippocampal synaptic transmission as well as behavioral and cognitive abnormalities such as increased anxiety, learning deficits, and impaired spatial memory [73]. Finally, Lim et al. [54] reported on a tg mouse model expressing human triple mutant tau under control of the murine thy-1 promoter. All of these mutants (G272V, P301L, and R406W) have been found in FTDP-17*T* kindreds [39]. Transgenic expression of this triple mutant at relatively low levels led to fibrillar, hyperphosphorylated tau pathology in neurons, in particular of cortical and

hippocampal regions. Transgene expression was low in the spinal cord, and no obvious motor deficits were observed up to 12 months [54].

In summary, in mouse lines engineered to express FTDP-17*T*-associated missense mutations, abundant filaments containing hyperphosphorylated tau protein developed, which was associated with neuronal cell loss. The fact that phosphorylation was observed in soluble tau points to the fact that hyperphosphorylation is likely to occur prior to filament assembly. Indeed, as demonstrated [63], an increase in tau phosphorylation in the soluble tau fraction may result in increased filament formation, indicating that phosphorylation of tau plays a contributory, if not causal role in the process of tau self-aggregation [63]. However, one crucial question that has not been answered yet in vivo is whether tau phosphorylation represents a primary event or occurs secondarily to the tau protein undergoing subtle conformational (pathological) changes.

Modeling glial pathology

Finally, two tg models where FTDP-17*T* mutations were expressed to model glial tau pathology, shall be briefly mentioned here. In the first approach, Götz et al. [30] expressed the human G272V mutation with a MoPrP promoter-autoregulatory tetracycline-dependent transactivator system and found transgene expression in neurons as well as oligodendrocytes. Although formation of filamentous tau associated with phosphorylation of the protein was observed, these mice did not develop any obvious neurological deficits [30]. In yet another P301L tg mouse strain with transgene expression regulated by an oligodendrocyte-specific promoter [2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP)], Higuchi et al. [36] demonstrated the mutant tau in CNS oligodendrocytes to become insoluble and filamentous at 15 months of age. In these mice, disruption of myelin and axonal degeneration occurred, and subsequent motor deficits developed. Finally, coiled body-like NFT formation in oligodendrocytes was also revealed in tg P301L mice (MoPrP promoter) described earlier [53, 55].

Double and triple transgenic mice as models for AD pathogenesis (Table 2)

Alzheimer's disease is neuropathologically characterized by two signature lesions, extracellular plaque-like deposition of Aβ as well as intracellular formation of NFTs containing hyperphosphorylated tau. Despite several decades of research efforts, it is still not known whether or not both pathologies are causally related to each other. According to the amyloid cascade hypothesis [32], and possibly in the reality of AD, they are in that Aβ is likely to function upstream to trigger tau pathology. Along these lines, Aβ

oligomers have recently been shown to impair proteasome activity, thereby enhancing tau accumulation [89]. Moreover, APP mutations are causally linked to early onset forms of AD, but no tau mutations have ever been linked to the disease. However, at least outside the context of AD, the general validity of the hypothesis can be questioned by a number of observations. First, in PSP, CBD, AgD, and FTDP-17*T*, neurodegeneration occurs in the absence of Aβ pathology. Second, it is interesting to note that tg mutant APP mice, despite showing extensive parenchymal and vascular amyloid pathology, do not develop robust tauopathy-related changes (see review by van Leuven [91]). Furthermore, it was recently reported that intraneuronal Aβ immunoreactivity may not be predictive of neurofibrillary tau pathology [94]. On the other hand, double tg mice, coexpressing mutant APP and the P301L tau mutation develop not only Aβ as well as tau pathology, but also display amyotrophy and severe motor problems [52]. In addition, triple tg mice (3xTg-AD) overexpressing mutant APP (harboring the Swedish mutation), the M146V presenilin-1 mutant, and the P301L mutant tau, exhibit a combined, progressive tau and amyloid pathology [66]. Synaptic dysfunction with deficits in long-term potentiation manifested prior to the occurrence of plaques and tangles [66]. Interestingly, in this model amyloid deposition precedes NFT formation, consistent with the amyloid cascade hypothesis [65]. Moreover, when proteasome function is blocked in 3xTg-AD mice at a pre-pathological stage, a marked increase in tau as well as Aβ accumulation ensues [89]. Furthermore, by intracerebrally injecting Aβ42 fibrils into P301L mice an increase in NFTs was triggered in the amygdala from where neuronal projections run to the injection sites, which is clearly indicative of a pathogenic link between Aβ and tau [28].

In another recent approach it was tested whether lowering the expression of endogenous tau could block Aβ-induced cognitive impairments. To this end, human APP transgenic mice [60] were crossed with tau knockout animals. Remarkably, ablation of endogenous murine tau prevents the Aβ-dependent behavioral deficits that typically develop in human tg APP mice, without having any measurable effects on amyloid plaque load [71]. Another important aspect of that same work [71] is that tau may have a role in regulating nerve cell activity and that lowering levels of tau expression might represent a strategy to inhibit hyperexcitability-induced damage of CNS neurons; the underlying molecular correlates of this excitotoxic tau effect remain to be clarified.

Therapeutic approaches

So far, available therapeutic approaches have only met with limited success, with only a few approved modalities that—

only transiently—slow cognitive decline [58]. Perhaps, unless the primary cause(s) triggering the neuronal cell death cascade in tauopathies are targeted, there are only modest chances to succeed in developing highly efficient therapeutic strategies capable of preventing or at least halting the vicious circle of neurodegeneration in these disorders.

According to the amyloid cascade hypothesis, reduction of the culprit Aβ, supposed to act upstream of tau, appears to be an obvious strategy. However, recent reports (e.g. [71]) indicate that reducing tau may be an equally warranted, effective, and possibly complementing approach.

A number of additional strategies are conceivable, one of them targeting abnormal tau phosphorylation. The longest adult tau variants contain almost 80 potential serine and threonine phosphorylation sites, although only about 30 of them seem to be functionally relevant under physiological conditions [12]. The phosphorylation status of tau can be modulated by the prolyl-isomerase Pin1 [56] which catalyzes the conversion between two distinct phospho(Ser/Thr)-Pro motif conformations, thereby perhaps also indirectly promoting tau dephosphorylation. Interestingly, Pin1 knockout mice exhibit progressive, age-dependent motor and behavioral deficits, accompanied by tau hyperphosphorylation, tau filament formation, and neuronal degeneration [56]. Apart from Pin1, in particular three kinases, GSK-3 [57], CDK5 [63], and ERK2 [47] have been associated with modulating tau phosphorylation in vivo. Of note, tg mice overexpressing GSK-3 display tau hyperphosphorylation, disrupted microtubules, and apoptotic neurons [57]. Interestingly, conditional expression of dominant-negative GSK-3 (unexpectedly, the constitutive GSK-3 knockout is embryonically lethal due to massive apoptosis in the liver [37]), leads to increased neuronal apoptosis and motor symptoms, both of which are suppressed when transgene expression is switched off [25]. In another experimental approach, the administration of an orally bioavailable, blood-brain barrier-penetrating small molecule inhibitor of ERK2 to tg P301L mice was effective not only at significantly reducing hyperphosphorylated tau but also in preventing the typical motor deficits in these animals. Remarkably, however, there was no concomitant reduction of NFTs, suggesting that tau cytotoxicity is not so much exerted by NFTs but may rather be caused by smaller tau aggregates. The prevention of hyperphosphorylation through inhibition of one of the tau-phosphorylating kinases may also point to a crucial pathogenic role of dysregulated tau phosphorylation in tauopathies [47].

However, CDK5, ERK2, and GSK-3 also function to regulate various important physiological pathways, necessitating the design of kinase inhibitors that effectively suppress tau hyperphosphorylation while showing only minimal toxicity through off-target effects. If successful, such kinase

inhibitors (recently reviewed in [59]) might yield a valuable adjunct to currently available treatment options.

In addition, administration of lithium-chloride, a drug traditionally used in the treatment of bipolar disorders, has recently been shown not only to inhibit GSK-3 activity but also to result in decreased tau pathology [62], which strongly correlated with markedly reduced axonal degeneration in P301L tg mice [64]. Other alternative (although currently more theoretic) approaches could be aimed at preventing the proteolytic cleavage-mediated generation of small, nucleation-promoting tau fragments through inhibition of specific proteases such as calpain or caspases [15, 35]. Stabilizing microtubules might represent another potential therapeutic avenue (for review see [87]). Agents with respective activity, in particular taxols such as paclitaxel are commonly used in cancer treatment (recently reviewed in [45]). As they act on dividing cells, adverse effects are hard to be avoided. Nevertheless, treatment of tg mice expressing the 3R/0N isoform of human wt tau with paclitaxel resulted not only in the restoration of fast axonal transport but also led to the improvement of motor deficits [99]. Finally, as Aβ oligomers were recently demonstrated to enhance tau accumulation through impaired proteasome activity, the proteasome appears to be another attractive target for therapeutic intervention [89].

Open questions

Admittedly, despite decades of intense research, many of the central questions underlying the pathogenic mechanisms in tauopathies still remain unanswered. In particular, the nature of the triggering event(s) initiating tau aggregation in vivo remains enigmatic. One of the questions that arises in that context is whether tau phosphorylation is a general prerequisite for filament formation and if so, how both processes are linked. To what extent are the nucleation-promoting effects mediated by smaller, proteolytically derived tau fragments important events in filamentous aggregation? If the amyloid cascade hypothesis is correct, what are the mechanisms of Aβ triggering tau filament assembly? Which species of tau are the toxic ones? Do filamentous tau aggregates even act in a protective way by promoting the sequestration of the toxic species? Finally, what is the role of inflammation related to the development of tau pathology? What is the basis for the AD-stage-specific spatio-temporal distribution of tau lesions?

Conclusions

Despite the fact that the available animal models cannot sufficiently mirror the complex pathogenesis of human tau-

opathies, they will continue to provide important new insights into the molecular pathways at play in these disorders. In particular, genetically engineered mouse models will continue to lead the way in tauopathy research. Rather than completely reflecting the entire pathogenic process with all its different aspects, they can be used to adequately recapitulate crucial aspects of these disorders. One of their particular strengths is that, even under the highly artificial conditions of transgene overexpression, our comprehension/understanding of tauopathies can be further expanded by studies of protein interactions as well as of induced protein modifications and their consequences. Last but not least, using worms and flies may pay off as well, e.g., in high-throughput drug-screens which may lead to the identification of agents that lend themselves for the designing of novel therapeutic strategies for these devastating diseases.

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