Modelling tau pathology in human stem cell derived neurons

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Abstract

Tau pathology is a defining characteristic of multiple neurodegenerative disorders including Alzheimer's Disease (AD) and Frontotemporal Dementia (FTD) with tau pathology. There is strong evidence from genetics and experimental models to support a central role for tau dysfunction in neuronal death, suggesting tau is a promising therapeutic target for AD and FTD. However, the development of tau pathology can precede symptom onset by several years, so understanding the earliest molecular events in tauopathy is a priority area of research. Induced pluripotent stem cells (iPSC) derived from patients with genetic causes of tauopathy provide an opportunity to derive limitless numbers of human neurons with physiologically appropriate expression levels of mutated genes for in vitro studies into disease mechanisms. This review discusses the progress made to date using this approach and highlights some of the challenges and unanswered questions this technology has the potential to address.

The importance of tau in neurodegeneration

The accumulation of hyperphosphorylated aggregates of the microtubule associated protein tau characterise a range of clinically diverse disorders collectively termed the tauopathies, including Alzheimer's Disease (AD) and frontotemporal dementia (FTD)(1). A direct link between tau dysfunction and neurodegeneration was confirmed by the discovery of mutations in the tau gene, *MAPT*, that cause FTD with tau pathology (2,3). Further genetic evidence linking *MAPT* to neurodegeneration is provided by the existence of two major haplotype blocks at the *MAPT* locus, H1 and H2. The H1 haplotype is associated with increased susceptibility to several sporadic tauopathies, including progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), as well as Parkinson's Disease (PD) (4–6). Mutations in *MAPT* do not cause AD, however there are several lines of evidence strongly supportive of a crucial role for tau in the AD pathological cascade. The extent of tau pathology is strongly correlated with symptom severity and neuronal cell loss (7). Further, experimental models have demonstrated that tau reduction is neuroprotective against amyloid, demonstrating an essential role for tau as a mediator of amyloid toxicity (8,9). Therefore, tau is an attractive therapeutic target for a broad range of neurodegenerative disorders and understanding the mechanisms linking tau to neurodegeneration is a research priority. A comprehensive review of the role of tau protein in health and disease can be found here (1).

A major limitation in our ability to understand the molecular mechanisms linking tau and neurodegeneration has been the availability of experimental models recapitulating key features of disease. For example, it has been difficult to generate rodent models that reliably recapitulate tau tangles and neuronal loss pathology, and certain aspects of tau biology that are important for neuronal health, notably its alternative splicing, are species-specific and experimental models do not recapitulate patterns observed in the adult human CNS. It is important to note that tau dysfunction and aggregation begin decades before the onset of clinical symptoms, by which time substantial neuronal cell loss has occurred, therefore treatment at pre-symptomatic stages is likely to be the most successful therapeutic strategy (10). Experimental models that allow the earliest stages of tauopathy to be dissected at the molecular level will be an important addition to the experimental toolkit. For the past 10 years, it has been possible to generate stem cells from terminally differentiated cell types, termed induced pluripotent stem cells (iPSC). As iPSC can be differentiated into any cell type of interest, including neurons, this technology can be used to generate limitless numbers of human cells, capturing the precise genetic make-up of the donor, making them a

powerful tool for the study of disease mechanisms (reviewed in 11,12). Here, I will discuss the progress and challenges of using this approach to create *in vitro* models of tauopathy.

iPSC as models of neurological disease

Our ability to generate *in vitro* models of neurological disease was revolutionised by the description of methods to reprogram terminally differentiated cells such as fibroblasts to pluripotency by the exogenous expression of pluripotency-associated transcription factors including (but not limited to) Oct4, Sox2, Klf4 and cMyc (13). The resulting iPSC are indistinguishable from embryonic stem cells across a number of key characteristics, including the ability to maintain a stable karyotype, expression of pluripotency genes, and their ability to be differentiated into any cell type of interest. Protocols for the directed differentiation into many specific neuronal subtypes exist, thus iPSC can provide a limitless supply of human neurons for the study of neuronal development and disease mechanisms (14). By taking the initial primary material from patients with a known genetic cause of disease, one can therefore generate “dementia in a dish” models to study the effects of these genetic mutations in a disease-relevant cell type and at physiological expression levels. These models have provided the field with an exciting opportunity to gain insight into disease mechanisms as well as developing humanised platforms for drug screening.

Modelling tau pathology in iPSC-neurons from AD

Much of what we know about the molecular mechanisms of AD has come from the study of rare genetic forms of the disease (familial AD, fAD) caused by mutations in the genes encoding the amyloid precursor protein (*APP*), and presenilin 1 and 2 (PSEN1 and PSEN2) which are components of the γ-secretase complex responsible for proteolytic cleavage of APP into its smaller fragments (15). All of these mutations lead to altered processing of APP, favouring the production of aggregation-prone Aβ peptides and amyloid plaque formation, and ultimately leading to downstream tau pathology. Many groups have now used iPSC-neurons with mutations in these three genes to gain insight into disease pathologies and disease mechanisms.

There are now numerous studies using iPSC-neuron models of fAD, which have universally demonstrated altered APP processing and the production and secretion of disease-associated Aβ peptides as a feature of these *in vitro* models, confirming the effects of fAD mutations are replicated faithfully in iPSC-neurons (16–23). Interestingly, unusual mutations such as the APP E693Δ variant resulted in intracellular Aβ accumulation as seen in patients, confirming this system can be used to reliably recapitulate specific mutation effects *in vitro*

(18). Human cortical neurons from individuals with Down Syndrome (who carry an extra copy of the APP gene) developed amyloid aggregates positive for the Thioflavin-S analog BTA1 (24). These robust read-outs of pathogenic mutations enable translation into medium and high-throughput platforms for drug screening, which has been successfully performed in this context to reveal modulators of APP processing (17,25).

A key question of iPSC models of fAD is whether they can be used to probe the links between amyloid and tau in the context of a human *in vitro* model. Elevated tau phosphorylation, mislocalisation and increased insolubility of tau has been described in numerous fAD iPSC models, confirming disease-associated changes to tau *in vitro*. Interestingly, a number of reports have described elevated tau protein levels in iPSC with APP coding or dosage mutations (19,20). This was apparent at the protein level but not the mRNA level, suggesting a previously unrecognised relationship between APP processing and tau proteostasis. iPSC have also been generated from sporadic AD patients (sAD) which account for the majority of AD cases. Elevated Aβ and tau phosphorylation was observed in some, but not all, of these neuronal cultures, highlighting the challenges of using this system to interrogate sporadic disease due to the inherent variability between different patient lines, driven largely by genetic background (18,23,26).

In addition to recapitulating amyloid and tau changes, iPSC-neurons from fAD patients have demonstrated a variety of cellular phenotypes including increased susceptibility to oxidative stress, abnormal endosomes and altered axonal transport (18,23,27). Whether any of these phenotypes can be rescued by modifying the levels or phosphorylation state of tau remains to be determined and will be an important area of future research. It is also important to note that these 2D culture systems have, so far, not generated both amyloid plaques and tau tangles within a single *in vitro* system.

3D models of AD

In addition to 2D, adherent neuronal cultures, there are several methods available for the generation of 3-Dimensional disease models. Recent reports have harnessed the intrinsic ability of stem cells to self-organise in non-adherent conditions to form cerebral organoids, which contain a diversity of neuronal cell types representative of different brain regions and display rudimentary architecture such as neuronal layering (28).

The cellular diversity and architecture offered by cerebral organoids confers several advantages over 2D adherent cultures. It should be noted, however that gene expression studies show cerebral organoids to closely resemble fetal brain, so although a powerful tool

for the study of development, to what extent organoids will recapitulate the pathophysiology of later-onset diseases remains to be determined (29). Raja and colleagues generated cerebral organoids from fAD iPSC with APP duplications or PSEN1 M146I and A264E mutations (30). These organoids showed a time-dependent increase in Aβ40 and Aβ42 when compare to controls, and amyloid accumulations were visible on tissue sections from these organoids. Further, this was accompanied by an increase in phosphorylated tau at T181 and S396 that was only apparent after 90 days in culture. Together these data support the utility of 3D organoids for AD research.

A separate study used human neuronal progenitor cells overexpressing APP and PSEN1 with fAD mutations to drive the formation of AD pathologies (31,32). These neuronal progenitor cells produce a 9 fold increase in Aβ40 and a 17 fold increase in Aβ42 after 6 weeks of differentiation in comparison to wild type cells. 3D neuronal cultures were generated from these lines by embedding in the 3D support matrix Matrigel, accelerating the accumulation of insoluble Aβ monomers and oligomers together with sarkosyl-insoluble, hyperphosphorylated, filamentous tau. It remains to be determined whether both amyloid and tau tangle pathologies can be recapitulated in a single cell culture system in the absence of overexpression of mutant fAD genes.

Chimeric models of AD

A major challenge in the field of AD has been the production of rodent models that recapitulate the hallmark plaque and tangle pathologies of AD, together with neuronal loss (33,34). A recent study generated chimeric models by transplanting human iPSC-derived neurons into either immunodeficient wild-type mice or immunodeficient APPPS1 mice, which contain human transgenes expressing APP_{Swe} and PSEN1 L166P, and develop age-dependent amyloid deposition (35). In wt mice the human neurons integrated without signs of degeneration, however in the AD model there was a progressive degeneration of the transplanted human neurons from 6 months onwards (36). Neighbouring mouse neurons remained unaffected, demonstrating selective vulnerability of human neurons. Interestingly, abnormal tau was detected in the human neurons by immunohistochemistry with MC1, an antibody that detects conformational alterations of tau associated with early stages of disease. However, neuronal death occurred in the absence of tangle formation, supporting the idea of dissociation between tau tangle formation and cell death.

Modelling tau mutations in iPSC neurons

The discovery of coding and splice-site mutations in *MAPT* that are causative of FTD confirmed the link between tau dysfunction and neurodegeneration (2,3). To date, over 40 *MAPT* mutations have been described (http://www.alzforum.org/mutations), with the majority located in exons 9-12 of *MAPT* which code for the microtubule binding repeats. The generation of experimental models featuring *MAPT* mutations provide insight into the mechanisms linking tau and neurodegeneration in FTD, but can be more broadly extrapolated to AD. As discussed below, several groups have used iPSC to successfully model *MAPT* mutations although there are several challenges associated with this approach, given that several aspects of tau biology relevant to disease are also developmentally regulated.

Tau splicing in iPSC-neurons

The alternative splicing of exons 2, 3 and 10 of the *MAPT* gene leads to the generation of six protein isoforms of tau in the adult human central nervous system (CNS). Exons 2 and 3 code for the N-terminal repeats of tau, and exon 10 codes for an extra microtubule binding repeat, and tau isoforms are characterised as either 3R (3 repeats) or 4R (4 repeats) accordingly (37,38). Thus, the six isoforms of tau are 0N3R, 0N4R, 1N3R, 1N4R, 2N3R and 2N4R. The levels of these are tightly controlled and appear to be critical for neuronal health: in normal circumstances the levels of 3R and 4R are approximately equal, but mutations in *MAPT* which disrupt this ratio (generally favouring an increase in exon 10 inclusion) are sufficient to cause FTD (39). A similar increase in 4R tau isoforms is also observed in the sporadic tauopathies PSP and CBD (40,41).

How an overproduction of specific tau isoforms can lead to neurodegeneration is an intriguing and unanswered question. A big challenge in this area has been the lack of appropriate experimental models to study disrupted tau splicing: it has been difficult to produce *in vitro* and *in vivo* models that recapitulate the complex nature of tau splicing seen in the adult human CNS. A human, neuronal model that correctly splices tau would be an important advance in this regard.

Human primary neurons have been shown to express and splice tau in a manner resembling the adult human CNS, however these have not been widely adopted due to the inavailability/ethical issues of using aborted fetal material (42). iPSC therefore offer an attractive opportunity to generate human neurons *in vitro* which would be predicted to recapitulate tau expression as seen the adult CNS. However, tau splicing is also subject to developmental regulation: the smallest (0N3R) is predominantly expressed during fetal stages with a post-natal switch to six tau isoforms (37). Several groups have now reported

that iPSC-neurons express predominantly the fetal tau isoform at early culture time-points, with other isoforms only expressed in substantial amounts after extended *in vitro* culture times (43–45). This is perhaps unsurprising in the context of genome-wide expression data demonstrating that iPSC-neurons strongly resemble fetal human brain (46). However, several *MAPT* mutations are located within alternatively spliced exons so this is an important point to consider when using iPSC-neurons for disease modelling.

Several protocols have now established an accelerated differentiation of iPSC into neurons, and for the direct conversion (trans-differentiation) of fibroblasts into neurons, which may promote retention of biological signatures of aging (47–49). It is interesting to speculate whether these accelerated neuronal differentiation paradigms will also accelerate the acquisition of mature tau isoforms. The splicing of *MAPT* can also be artificially controlled to favour 3R or 4R isoform expression using trans-splicing, resulting in altered axonal transport of cargo including APP (27).

Importantly, many groups have now shown that exonic and intronic *MAPT* mutations associated with altered tau splicing are able to disrupt tau splicing *in vitro*. iPSC-neurons with the N279K, 10+16, and 10+14 *MAPT* mutations all lead to an overproduction of 4R tau isoforms as seen in patients (43,45,50).

Neuronal phenotypes in MAPT mutation neurons

In spite of the developmental regulation of tau, iPSC models have shown multiple phenotypes relevant to FTD pathogenesis. An increase in insoluble tau was detected in neurons with the 10+14 intronic mutation, and the A152T and R406W coding variants in tau (51,52). The best-characterised function of tau is its role as a microtubule associated protein, prompting several groups to examine whether intracellular trafficking and axonal transport could be disrupted in iPSC-neurons with *MAPT* mutations. Altered splicing and coding mutations in tau resulted in altered vesicle trafficking and an altered distribution of mitochondria within neurons (45,50). Mitochondrial dysfunction and reduced ATP production was observed in neurons with the 10+16 mutation, which could be related to disrupted mitochondrial transport (53). Dysregulated calcium signalling and an upregulation in markers of cell stress (including mitochondrial stress, altered endosome/lysosome composition, increased markers of stress granules) confirm the ability of tau mutations to induce neuronal dysfunction *in vitro* (50,51,54). Interestingly, neuronal connectivity may be disrupted by tau mutations: both splice-site and coding mutations in *MAPT* lead to an accelerated acquisition of electrical maturity *in vitro* (45).

Tau seeding and spread in iPSC-neurons

In AD, tau pathology spreads between connected regions of the brain in a temporal and spatially predictable manner that correlates with symptom severity and the extent of neuronal loss. An area of intense research is now focussed on understanding the trans-synpatic spread of tau and the ability of pathological tau species to seed aggregation in recipient neurons, and human iPSC-neurons will be a powerful tool in these investigations. Truncated tau species lacking the C-terminus of the protein is readily detectable in conditioned media from iPSC-neurons (55), and a recent study showed tau release from iPSC-neurons is activity dependent (56). Pre-formed tau aggregates were able to induce tau aggregation and hyperphosphorylation in iPSC-neurons transduced with the pro-aggregation P301L mutant, in an assay that has been successfully scaled into 384-well format that can be used to screen for inhibitors of tau aggregation(57,58).

Conclusion

Since the first description of iPSC 10 years ago, numerous groups have used this technology to generate *in vitro* models for AD and FTD. These have demonstrated the power of this approach to create “disease in a dish” models that capture key aspects of AD and FTD pathology, including altered APP processing, tau splicing, phosphorylation and aggregation. These models have provided insight into novel disease mechanisms in addition to providing humanised platforms for drug-screening. This review has focussed on iPSC models from patients with a known genetic cause of tauopathy. The advent of genome-wide association studies (GWAS) has identified many genetic risk variants, investigation of the molecular mechanisms linking these to disease pathology will be an exciting area of future research. There are several challenges to overcome in the use of iPSC as models for adult-onset neurodegenerative disease, including the developmental maturity of the resulting neurons, reductionist approach of studying isolated cell types, and the variability between iPSC from different donors. New protocols for accelerated differentiation, co-culture of multiple cell types, and generation of isogenic lines using CRISPR will enable the generation of robust models with greater physiological relevance. In this rapidly moving field, it is clear that iPSC will continue to contribute greatly to our enhanced understanding of tauopathy.

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