Deciphering tau-related dementia using human iPSC lines: electrophysiological perspectives of future studies

Olga Kopach*

To date, no disease-modifying treatment or cure is available for dementia. This disorder is becoming more common as the global population ages. There has been over several decades of extensive research focusing on how the pathology develops and progresses causing memory loss, brain damage, and eventually death – it provides the field with a deep understanding of what proteins, peptides, and signaling molecules contribute to neurodegeneration at the molecular, genetic, and cellular levels. The problem is, however, that there is a wide range of dementia types. A given disease can span heterogeneous clinical syndromes with diverse symptomatology, no matter whether it is "senile dementia" or an early-onset form; moreover, it encompasses the mixed features of many syndromes in later stages of the disease. In confirmation of this, animal models, despite being purposely designed and widely used in dementia research, usually do not fully replicate neuropathological profiles that match pathological changes found in the human brain, much less in cognitive and intellectual decline as it occurs in patients. This made it hitherto insurmountable for scientists to conceptualize the basis underlying nerve cell dysfunctions in a range of dementias.

The latest advances in generating patient-specific neural cells from induced pluripotent stem cells (iPSC) have enabled studies directly in live human cells. It opened herein an avenue for investigating the human cell pathology at the cellular/subcellular level, having known the history of dementia and clinical symptoms. Furthermore, patient-specific iPSC lines provide the most reliable platform for drug screening, which helps to facilitate tailored management of the disease-modifying treatments, including pharmacological and non-pharmacological (genetic) strategies. There were exceptional studies using electrophysiology in human iPSC-derived neurons in autism, Down syndrome, Dravet syndrome, Alzheimer's and Parkinson's diseases. However, physiological studies of iPSC-derived nerve cells are much less common and more challenging. Our electrophysiological studies have recently revealed the neuropathological phenotype of cortical neuronal dysfunction in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) (Kopach et al., 2020, 2021; Esteras et al., 2021). This disease, caused by a mutation in the gene encoding the microtubule-associated protein tau (*MAPT*), has been the second most common form of early-onset dementia. Using patch-clamp electrophysiology and live-cell fluorescent imaging, we documented the impaired membrane properties and intrinsic excitability of iPSC-derived cortical neurons, accompanied by synaptic dysfunction, changed intracellular calcium signaling, and dysfunctional mitochondria in cells derived from FTDP-17 patient samples. The knowledge of impaired biophysical traits and aberrant neuronal activity advances our understanding of how pathogenic tau damages neuronal function, causing dementia.

Phenotype of human cell neuropathology in 4-repeat (4R) tau-induced dementia: Tauopathy is an umbrella term that encompasses a wide range of neurodegenerative disorders (more than 20 clinicopathological phenotypes). This includes Alzheimer's dementia, several forms of frontotemporal lobar degeneration – such as FTDP-17, Pick's disease, globular glial tauopathy – subtypes of parkinsonism (clinically similar to Parkinson's disease), argyrophilic grain dementia, movement disorders (corticobasal degeneration, progressive supranuclear palsy), among others. Despite a variety of clinical and genetic profiles (which may include, or may not, the loss of memory and cognitive skills), the abundant, highly toxic deposition of the protein tau is a hallmark of tauopathies. The pathogeny originates from mutations or pathological triggers over an individual's life those cause tau to become sticky, affecting other proteins and cellular regulatory systems that result in widespread cell death. The primary molecular mechanism, as established to date, includes self-aggregation of hyperphosphorylated tau followed by conformational changes in microtubule dynamics.

There are six tau isoforms expressed in the adult human brain as a result of alternative splicing of exons 2, 3, and 10 of the gene 17q21. The isoform structure differs substantially in their tubulin-binding domains, varying between 3-repeat and 4R isoforms of tau. The correct splicing (balanced 3-repeat/4R ratio) is required for normal neuronal function, while pathological conditions may lead to abnormal expression and aggregation of 4R tau: several *MAPT* mutations cause overproduction of 4R tau and trigger neurodegeneration with dementia. Our recent studies (Esteras et al., 2021; Kopach et al., 2021) have shown that the inclusion of exon 10 via genetically engineered intronic *MAPT* 10+16 mutation in healthy donor iPSC lines – hence overexpression of the 4R tau associated with tau hyperphosphorylation at different phosphorylation sites (Ser396/Ser404 and Thr181) (Verheyen et al., 2018) – resulted in neuronal dysfunction and pathological excitability of the cells. This genetically engineered approach effectively reproduced the pathophysiology of iPSC-derived neurons revealed in FTDP-17 patient samples with the *MAPT* 10+16 mutation (Kopach et al., 2020). Our studies have further discovered the mechanism of such impairments (**Figure 1**): it engages tau-damaged mitochondria which begin generating highly reactive by-products – reactive oxygen species (ROS). The latter causes heavy oxidation of numerous proteins, coupled with converted structural entities, and leads to dysfunctional membrane receptors/ion channels, such as voltage-gated sodium ion channels (Na_v), receptors for excitatory neurotransmitter glutamate: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. As part of the functional consequences, human cells display altered membrane properties (i.e. depolarized resting membrane potential and increased resistance), abnormal intrinsic excitability, and aberrant firing (related to changed channel properties of Na_v and altered surface expression of the receptors/ion channels: in particular, decreased $Na_v1.6$ channel subtype, while increased GluA1-containing AMPAR and NR2B-containing NMDAR). The increased expression of Ca^{2+}-permeable glutamate receptors leads to augmented Ca^{2+} entry through AMPAR and NMDAR, hence severely disturbed intracellular Ca^{2+} dynamics (both in the soma and dendrites) upon neuronal depolarization (**Figure 1**; Kopach et al., 2020, 2021; Esteras et al., 2021). This chain of neuropathological events includes synaptic dysfunction followed by abnormal network activity and ultimately neuronal cell death, causing brain dysfunction and dementia.

Is ROS-induced pathological signal transduction generic for tauopathies? Changes in the redox status by generated

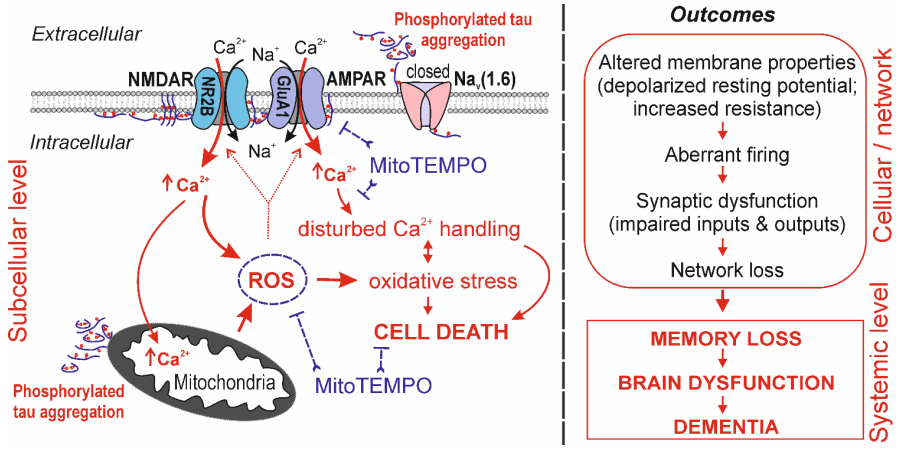

Figure 1 | **Schematic illustration of the pathogenic cascade of intracellular events induced by overexpression of 4R tau in human iPSC-derived cortical neurons (left), causing a chain of neuronal dysfunctions and neuropathology (right) in frontotemporal dementia and parkinsonism linked to chromosome 17.**

The left panel shows the cascade at the subcellular level triggered by pathogenic hyperphosphorylated 4R tau, making protein aggregates inside the cytosol, within the membranes, and spreading extracellularly – this affects (i) mitochondria that begin producing excessive ROS, causing heavy oxidation of various proteins and (ii) functional membrane receptors/ion channels. In particular, the surface expression of GluA1-containing AMPAR and NR2B-containing NMDAR increases, causing augmented Ca^{2+} entry through the receptor and disturbed intracellular Ca^{2+} handling (red arrows). The resulting oxidative stress and Ca^{2+}-induced excitotoxicity lead to neuronal cell death. The right panel lists the functional outcomes at (i) neuronal level, such as changes in the membrane properties, causing aberrant neuronal excitability, impaired synaptic function, loss of synaptic connections, and (ii) systemic level, by experiencing memory difficulties, brain dysfunction, and eventually severe dementia. Note that treatment of human iPSC-derived neurons with mitochondrial antioxidant MitoTEMPO restored the ROS level together with the augmented membrane currents through AMPAR and NMDAR and pathological Ca^{2+}-rise in the cells derived from the FTDP-17 patient samples (blue lines) – this ultimately prevented neuronal cell death (left panel). AMPAR: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; iPSC: induced pluripotent stem cells; NMDAR: N-methyl-D-aspartate receptors; ROS: reactive oxygen species; MitoTEMPO: a mitochondrial superoxide scavenger.

ROS are inevitably linked to cell function. This is especially characteristic for neurons that have high metabolic rates due to continuous activity and high-frequency patterns of discharge (common for cortical neurons), thus, greater amounts of generated ROS. However, excessive ROS production, if not counteracted with antioxidants (either enzymatic or non-enzymatic, or both), may cause an abundance of unchecked ROS and oxidative stress, resulting in pathogenesis that might severely exacerbate an impact initiated by the pathogenic trigger on its own. Exceeding NMDAR activation can be an alternative source of unchecked ROS, contributing to neuronal cell death (Brennan et al., 2009). If so, a level of ROS-induced oxidation could be reversed, at least to some extent, to preserve innate protein structures from damage, hence rescue the function. We have utilized such a regulatory switch by using mitochondrial antioxidants for the treatment of human iPSC-derived cortical neurons in FTDP-17. Our data showed (Esteras et al., 2021) that mitochondrial antioxidants restored both AMPAR- and NMDAR-mediated currents and recovered the unbalanced Ca^{2+} signals, in either patient samples or genetically engineered iPSC lines overexpressing 4R tau – this all in all reduced neuronal cell death (**Figure 1**).

An important question however remains whether such a redox-switched pathological chain of signaling events underlies the neuropathology in other tauopathies. Could it be the underlying mechanism of neurodegeneration associated with a particular type of dementia? Or can it present the basis of neuronal dysfunction elsewhere in tau-related pathology? A notable clinicopathological example could be a group of 4R tau-induced movement disorders which are, despite being tauopathy, not linked to memory loss and cognitive impairments. On the other hand, glutamate receptor dysfunctions were found to be the cause of synaptic impairments in tauopathies associated with memory loss (Olivares et al., 2012; Decker et al., 2016) and motor degeneration-spectrum disorders (Radzicki et al., 2016). This can make it a focus of further research to examine whether the tau-induced redox switches can serve as a generic mechanism of human cell dysfunction across heterogeneous tauopathies.

"Matured" iPSC-derived neurons – challenges and significance: One of the most important issues while working on iPSC lines has been determining the time when iPSC-derived neurons mature "proper" biophysical properties. In iPSC cultures, it has been routine to apply protein/receptor marker expression analysis; however, this approach does not confirm whether receptors (ion channels) are functional and whether differentiated cells exhibit a reliable neurophysiological activity. It must be tested alongside the neuronal function, such as cells' capability to generate action potentials, maintain consistent frequency discharge upon stimulation, network activity, among others. Leveling out the maturation of biophysical properties of the differentiated cells in control conditions is essential before concluding phenotypic profiles in the pathology (Kopach and Pivneva, 2019). In iPSC lines, we performed patch-clamp recordings at various time points to trace the anticipated maturation of biophysical properties of human iPSC-derived cortical neurons and found out that it takes ~150 days *in vitro* (Kopach et al., 2020). A similar time period was found for genetically-engineered iPSC lines (Kopach et al., 2021), which we used in conjunction with the age-matched control (parental lines with wild-type tau). For quantitative comparisons of any parameter of interest, it is imperative to test the age-matched cells across experimental cohorts (e.g., control *versus* patient samples). Our electrophysiological data validated that human iPSC-derived neurons met expectations for functional studies at the sub-cellular, cellular, and neuronal network levels when used at late neurogenesis. Given that intrinsic properties determine key aspects of neuronal function and behavior, it appears that human iPSC-derived neurons level out their intrinsic excitability up to a common neurophysiological level over extended neurogenesis (up to ~5–6 months) that is far longer than previously assumed from immunochemical analyses.

In addition to electrophysiological parameters (e.g., capacitance), the morphological assessment is of great use. It helped to navigate the time-dependent neurogenesis by quantifying morphological parameters of iPSC-derived neurons, such as the diameter of soma, a relative proportion of astroglia between control and demented groups (Kopach et al., 2020; 2021), others.

For the genetic and tau protein backgrounds, it has been confirmed the cells' ability to replicate mutations linked to tau pathogenesis during *in vitro* neurogenesis as well as developmental changes in tau splicing replicated in iPSC-derived neurons over long-term maintenance (Sposito et al., 2015; Verheyen et al., 2018). In human iPSC lines, there were high levels of tau phosphorylation during development, detected at multiple epitopes (Ser396/Ser404 and Thr181 phosphorylation sites) that are linked to the protein aggregation into tangles in the brain.

Some remarks regarding future directions: Summarizing, patient-specific iPSC lines

provide a valuable source of human neural cells that: firstly, effectively meet the ethics and safety requirements due to the reprogramming of somatic cells into iPSC; secondly, firmly replicate genetic and phenotypic profiles over cell differentiation; and thirdly, enable thus to examine live human cells "in a dish". Using advances in the technology of genome editing (e.g., CRISPR/Cas9-based correction of gene mutations), a new milestone in human cell research has emerged, facilitating up-to-date therapeutic strategies against human cell neurodegeneration that causes dementia.

Long-term maintenance of iPSC lines in adherently expanded 2D cultures has been common, however, growing iPSC assemblies (3D cultures) appears to provide more physiologically favorable conditions. Emerging protocols, which are actively being developed to build up iPSC organoids, enable a multilayer microenvironment that facilitates the maturation of iPSC-derived neurons. Such a valuable advantage is essential while aiming at examining cellular dysfunction in late-onset dementias (typical and atypical Alzheimer's disease). Studying age-related neurodegeneration requires an *in vitro* model of rapid neuronal development and maturation as compared to long-term 2D cultures. In addition, in Alzheimer's disease pathogenesis, the age-dependent profiles of pathogenic tau can span patterns of amyloid-β peptides – a feedback loop to exacerbate each other by prion-like mechanisms. Likewise, fibrillar tau and α-synuclein aggregates often exist at the neuronal plasma membrane, contributing very likely at an age-dependent manner to Alzheimer's, Parkinson's, and other tau-related diseases. In such cases, modeling the disease with organoids would ensure a more reliable assessment of varied cellular phenotypes. One of the other advantages of iPSC organoids is the growing of different neural cell types for further testing within one entity. It presents a focus of following studies, especially taking into consideration that in patients with dementia, both astrocytes and oligodendrocytes (aside from neurons) contain filamentous tau inclusions. Notably, tauopathies associated with movement disorders, such as progressive supranuclear palsy and corticobasal degeneration, also exhibit glial pathology. Given that dysfunction of glial cells affects neuronal function and contributes largely to impaired learning and memory, it is essential to thoroughly investigate phenotypes of neuronal and glial cell dysfunction, with possible overlapping between them, across the heterogeneous group of 4R tauopathies.

The author thanks Prof. Andrey Y Abramov from UCL Queen Square Institute of Neurology, London, UK for valuable comments and discussion.

Olga Kopach*
Queen Square Institute of Neurology, University College London, London, UK
***Correspondence to:** Olga Kopach, PhD, DSc, o.kopach@ucl.ac.uk.
https://orcid.org/0000-0002-3921-3674 (Olga Kopach)

Date of submission: September 28, 2021
Date of decision: November 20, 2021
Date of acceptance: December 12, 2021
Date of web publication: April 29, 2022

https://doi.org/10.4103/1673-5374.339482
How to cite this article: *Kopach O (2022) Deciphering tau-related dementia using human iPSC lines: electrophysiological perspectives of future studies. Neural Regen Res 17(12):2672-2674.*
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Open peer reviewer: *Mette Habekost, Lund University, Sweden.*
Additional file: *Open peer review report 1.*

References

Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH, Swanson RA (2009) NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. Nat Neurosci 12:857-863.

Decker JM, Krüger L, Sydow A, Dennissen FJ, Siskova Z, Mandelkow E, Mandelkow EM (2016) The Tau/A152T mutation, a risk factor for frontotemporal-spectrum disorders, leads to NR2B receptor-mediated excitotoxicity. EMBO Rep 17:552-569.

Esteras N, Kopach O, Maiolino M, Lariccia V, Amoroso S, Qamar S, Wray S, Rusakov DA, Jaganjac M, Abramov AY (2021) Mitochondrial ROS control neuronal excitability and cell fate in frontotemporal dementia. Alzheimers Dement. doi: 10.1002/alz.12394.

Kopach O, Pivneva T (2019) Cell-based therapies for neural replacement strategies in stroke-related neurodegeneration: neurophysiological insights into stem progenitor cell neurogenesis within a host environment. Neural Regen Res 13:1350-1351.

Kopach O, Esteras N, Wray S, Rusakov DA, Abramov AY (2020) Maturation and phenotype of pathophysiological neuronal excitability of human cells in tau-related dementia. J Cell Sci 133:jcs241687.

Kopach O, Esteras N, Wray S, Abramov AY, Rusakov DA (2021) Genetically engineered MAPT 10+16 mutation causes pathophysiological excitability of human iPSC-derived neurons related to 4R tau-induced dementia. Cell Death Dis 12:716.

Olivares D, Deshpande VK, Shi Y, Lahiri DK, Greig NH, Rogers JT, Huang X (2012) N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. Curr Alzheimer Res 9:746-758.

Radzicki D, Liu E, Deng HX, Siddique T, Martina M (2016) Early impairment of synaptic and intrinsic excitability in mice expressing ALS/dementia-linked mutant UBQLN2. Front Cell Neurosci 10:216.

Sposito T, Preza E, Mahoney CJ, Seto-Salvia N, Ryan NS, Morris HR, Arber C, Devine MJ, Houlden H, Warner TT, Bushell TJ, Zagnoni M, Kunath T, Livesey FJ, Fox NC, Rossor MN, Hardy J, Wray S (2015) Developmental regulation of tau splicing is disrupted in stem cell-derived neurons from frontotemporal dementia patients with the 10 + 16 splice-site mutation in MAPT. Hum Mol Genet 24:5260-5269.

Verheyen A, Diels A, Reumers J, Van Hoorde K, Van den Wyngaert I, van Outryve d'Ydewalle C, De Bondt A, Kuijlaars J, De Muynck L, De Hoogt R, Bretteville A, Jaensch S, Buist A, Cabrera-Socorro A, Wray S, Ebneth A, Roevens P, Royaux I, Peeters PJ (2018) Genetically engineered iPSC-Derived FTDP-17 MAPT neurons display mutation-specific neurodegenerative and neurodevelopmental phenotypes. Stem Cell Reports 11:363-379.

P-Reviewer: Habekost M; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y