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Towards mechanism-based tau-targeted therapies

Lidia Bakota, Roland Brandt*

Tau plays a crucial role in several neurodegenerative diseases, collectively referred to as tauopathies. Therefore, targeting potential pathological changes in tau could enable useful therapeutic interventions. However, tau is not an easy target because it dynamically interacts with microtubules and other cellular components, which presents a challenge for tau-targeted drugs. New cellular models could aid the development of mechanism-based tau-targeted therapies.

Tauopathies are a group of neurodegenerative diseases characterized by increased phosphorylation and aggregation of the microtubule-associated protein tau (Arendt et al., 2016). Certain tauopathies, such as Frontotemporal Dementia with Parkinsonism linked to Chromosome 17 (FTDP-17), are caused by mutations in the *tau* gene, while in the most common tauopathy, Alzheimer's disease (AD), neurofibrillary tangles composed of tau aggregates are accompanied by the presence of senile plaques composed of amyloid-beta peptide. However, even in AD, tau pathology correlates much better with progressive neurodegeneration than the formation of senile plaques, indicating that alterations in tau protein are pivotal in the neurodegeneration process. Thus, focusing on tau and its pathological modifications could offer a promising strategy to mitigate neurodegenerative progression.

Yet, tau is not an easy target. The tau gene arose through a gene duplication event early in vertebrate evolution from a common precursor gene with microtubule-associated protein 2 (MAP2), another microtubule-associated protein (Sundermann et al., 2016). While MAP2 is predominantly present in the somatodendritic compartment of mature neurons, tau is enriched in the axon. Sequencing data indicates that all vertebrates possess at least one tau gene, highlighting its essential role across species. Therefore, it was surprising that knocking out tau in mice did not result in major phenotypic changes and the animals developed functional axons (Harada et al., 1994). This also implied that contrary to the traditional view, the main role of tau is not to stabilize microtubules (Baas and Qiang, 2019). Consequently, it cannot be assumed that the loss of tau function due to tau aggregation in disease directly leads to a breakdown of the axonal microtubule array, as is still portrayed in many schematic representations of disease mechanisms.

What then is the biological activity and function of tau? In the axon, tau exhibits a highly dynamic interaction with microtubules, which we termed "kiss and hop" (Janning et al., 2014). While more than 80% of tau is present on microtubules at any given time, the residence time of a single tau molecule at a microtubule-binding site is only about 40 ms in cultured neurons. Despite this short interaction time, tau is still able to modulate microtubule dynamics. The "kiss and hop" interaction is likely crucial for tau's ability to modulate microtubule dynamics without affecting vital axonal transport processes that also occur on axonal microtubules. Indeed, modifications of tau that increase its residence time on microtubules impair the efficiency of axonal transport, leading to neuronal atrophy (Conze et al., 2022). In addition to the changes that could be a direct consequence of the microtubule interaction activities of tau, several more subtle changes have been observed in mice lacking tau protein, including alterations in synaptic plasticity, memory formation, and anxiety-related behavior, suggesting additional functions of tau (Brandt et al., 2020). Indeed, in addition to the microtubule-binding region (MBR), tau carries a long N-terminal projection region containing intrinsically disordered regions. Functionally, intrinsically disordered regions could be relevant because they offer a large interaction surface, thus allowing the binding of many interaction partners. Indeed, several binding partners of tau's N-terminal projection region have been identified, including plasma membrane components and members of various signaling cascades.

The most obvious approach to prevent tau-dependent neurodegeneration would be to inhibit or at least reduce disease-related changes in tau protein, such as increased phosphorylation or the formation of tau aggregates. However, several aspects regarding the potential physiological role of tau need to be taken into account (**Figure 1**). Regarding tau phosphorylation, most of the sites showing increased phosphorylation (hyperphosphorylation) during disease are highly conserved serine and threonine residues flanking tau's MBR (Trushina et al., 2019). These include binding sites for commonly used antibodies to detect the hyperphosphorylated state of tau protein, such as the AT8 antibody (phosphorylation at Ser202/Thr205) or the PHF1 antibody (phosphorylation at Ser396/Ser404). However, the interaction of tau with microtubules is also regulated by phosphorylation at regions flanking the MBR of tau, and in many cases, reduced phosphorylation of these sites leads to increased interaction of tau with microtubules. As mentioned above, the highly dynamic interaction of tau with microtubules is required for tau not to disrupt axonal transport. Therefore, reduced phosphorylation below physiological levels may generate hyperactive tau proteins that disrupt axonal transport and cause neuron degeneration. Furthermore, tau is not the only target for kinases and phosphatases and affecting phosphorylation-modulating proteins can also affect the phosphorylation states of many other target proteins, potentially having functional consequences.

Undesirable consequences could also be caused by approaches aimed at reducing tau aggregates. The MBR of tau is involved in both binding to microtubules and the formation of tau aggregates. Drugs that bind to the MBR of tau and thus prevent the formation of tau aggregates may therefore also disrupt the microtubule interaction of tau and thus the physiological regulation of microtubule dynamics. Furthermore, the MBRs of tau and MAP2 are highly conserved, so drugs that bind to the MBR of tau most likely also affect the interaction of MAP2 with microtubules, which may lead to dendritic defects. Additionally, it is still controversial what constitutes the bioactive and toxic species of the aggregation process. Experimental results suggest that soluble tau aggregates are the cause of toxicity (Lasagna-Reeves et al., 2011). In this case, higher tau aggregates such as the neurofibrillary tangles may represent nothing more than a kind of garbage bin where pathological tau is collected in an inactive form. In such a scenario, drugs that dissolve higher tau aggregates could even accelerate the degenerative process by increasing the amount of toxic soluble tau species.

Recent approaches aim to reduce the overall level of tau protein in patients with mild AD using an antisense approach (Mummery et al., 2023). This seems to be justified by the observation that the overall amount of tau increases during the development of AD and pathological consequences could then be attenuated by counteracting this change. However, as mentioned above, tau is a multifunctional protein that can affect many neuronal processes and signaling mechanisms in

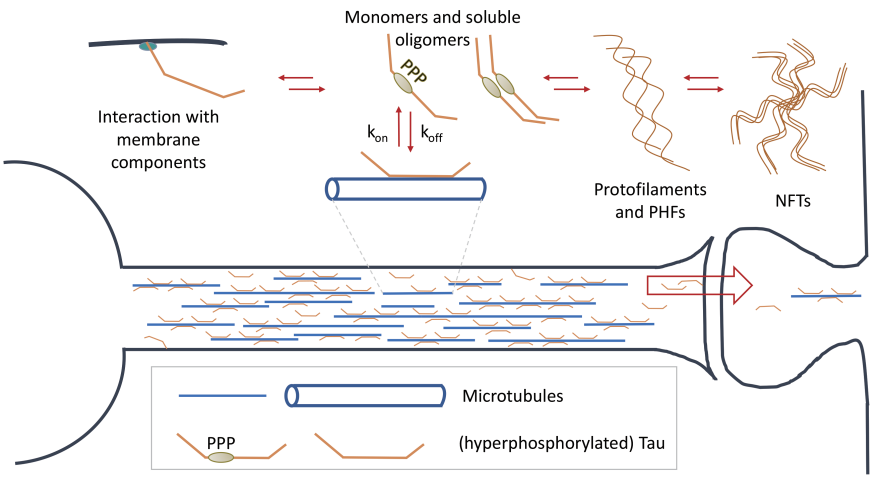

Figure 1 | **Dynamics of the tau protein in healthy and diseased states.**
Reactions whose modulation could be therapeutically useful are marked in red. See text for details. Hyperphosphorylation at the proline-rich region is marked by "PPP". k_{on}, k_{off}: On- and off-rate constants of the tau-microtubule interaction; NFTs: neurofibrillary tangles; PHFs: paired helical filaments.

subtle ways. Therefore, a general reduction of tau protein below a critical level could have adverse effects, for example in terms of memory processes or mood disorders.

What conclusions can be drawn from this for the development of mechanism-based tau therapies (**Figure 1**)? (a) Successful drugs must reduce tau hyperphosphorylation and reduce tau aggregation without affecting the dynamic interaction of tau with microtubules and possible other tau activities. (b) Successful drugs targeting tau aggregates should prevent the formation of new aggregates but not dissolve existing ones. (c) Modulation of a multifunctional protein such as tau could benefit from a polypharmacological drug approach.

A first consequence is the need to establish neuronal models that allow quantifying both tau aggregation and the interaction of tau with axonal microtubules. Particularly helpful is the identification of tau mutations that increase tau aggregation, such as the single amino acid deletion mutant TauΔK280 reported in tauopathies (Momeni et al., 2009). Indeed, we were able to show that recombinant TauΔK280 exhibited more than 50% increased aggregation compared to wild-type tau in cell-free aggregation assays, and long-term expression of this construct led to progressive tau amyloid formation in primary neurons (Pinzi et al., 2024). Furthermore, developments in single-molecule tracking and quantitative live-cell imaging allow the determination of the kinetics of the tau-microtubule interaction in axons of living neurons (Janning et al., 2014). A combination of these approaches revealed that the aggregation-prone TauΔK280 had reduced interaction with microtubules compared to wild-type tau, consistent with the interpretation that aggregate formation reduces the amount of tau protein available for binding to microtubules. Indeed, the progressive formation of tau aggregates in primary neurons also led to a progressive decrease in the interaction of tau with axonal microtubules (Pinzi et al., 2024). Therefore, expression of pathologically aggregation-prone tau combined with live-cell imaging to assess the extent of tau microtubule interaction may provide a cellular model to identify drugs that both reduce tau aggregation and restore physiological tau-microtubule interaction.

As a proof of concept, we used this neuronal model to screen a panel of small molecules predicted to bind to tau protein. Indeed, one of the compounds increased the binding of aggregation-prone tau to microtubules similar to wild-type tau levels in model neurons, suggesting that it reduced pathological tau aggregation in the cells. Accordingly, it also reduced tau amyloid formation in primary neurons after long-term expression (Pinzi et al., 2024).

Such a model allows identifying candidate factors that inhibit tau aggregation and restore physiological tau-microtubule interaction. However, as described above, a successful drug candidate should prevent tau aggregation without affecting existing neurofibrillary tangles to avoid increased formation of potentially toxic soluble tau aggregates. This activity can be assessed *in vitro*; our findings indicate that this small molecule candidate reduced tau aggregate formation in cell-free assembly assays but did not dissolve pre-existing tau aggregates or insoluble paired helical filaments isolated from AD patients. Accordingly, the compound also diminished the formation of tau amyloids in cells without causing a decrease in their overall levels.

In tauopathies, the formation of pathological tau aggregates is accompanied by increased phosphorylation at selected sites. A drug candidate that modulates both aspects, tau aggregation and tau phosphorylation, could therefore be superior to more mechanistically restricted candidates. Experimentally, this would require detecting the kinome activity of neurons by systematically determining the effect of potential drug candidates on gene expression and protein phosphorylation. This can be done by proteomic and phosphoproteomic analysis of the cells, which would allow, on the one hand, identifying changes in tau phosphorylation, and, on the other hand, by kinase enrichment analysis to link the identified phosphorylation sites to reduced activity of the kinases most likely responsible for the reduced protein phosphorylation. Indeed, a corresponding analysis of our drug candidate showed that it reduces phosphorylation of tau in the proline-rich region of tau, which is involved in regulating tau aggregation and tau-microtubule interaction, and induces reduced activity in tau kinases such as glycogen synthase kinase-3β, which has previously been implicated in mediating tauopathies (Pinzi et al., 2024).

Although the model is promising, there is still room for improvement. First, the cellular model is based on the exogenous expression of a human tau construct in rodent cells, meaning that the cells still express a background of endogenous rodent tau. The sequence and expressed isoforms of rodent tau differ from human tau, which may be relevant for the development of tauopathies, and rodent tau may modulate the effect of the exogenously expressed human tau construct. In addition, human neurons may respond differently than rodent cells, as they also have a very different timeline of their development. Therefore, the neuronal model could be improved by using human neurons differentiated from patient-derived induced pluripotent stem cells with pathogenic tau mutations, for example from FTDP-17 patients. However, even with such an improvement, another limitation arises: tauopathies are age-related diseases and increasing age is the main risk factor. Currently, induced pluripotent stem cell-based models do not reflect aged neurons and also the culture time cannot be extended indefinitely. Of course, it must also be taken into account that dissociated neuronal cultures will never reflect the complexity of interactions between neurons and non-neuronal cell types that occur in a real brain, and it is still controversial why tau pathology develops stereotypically in selected brain regions. In this respect, cell-to-cell tau spread could also play an important role (**Figure 1**). If this is the case, this aspect would also have to be taken into account by a successful therapy.

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迈向基于机制的 tau 靶向治疗
文章特色分析

一、文章重要性

1. 聚焦关键病理蛋白：

Tau 蛋白是多种神经退行性疾病（如阿尔茨海默病、额颞叶痴呆等）的核心病理蛋白，其异常磷酸化和聚集与神经元退化密切相关。文章强调 tau 病理比 Aβ 斑块更能反映疾病进展，因此靶向 tau 具有重要的治疗潜力。

2. 揭示治疗挑战：

文章指出 tau 蛋白功能复杂，不仅与微管动态相互作用，还参与信号转导、突触可塑性等过程，因此直接干预 tau 可能带来副作用，如影响轴突运输或记忆形成。

3. 推动机制导向治疗发展：

文章提出必须基于对 tau 生理和病理机制的深入理解来设计药物，避免“盲目”干预，这为精准神经治疗提供了理论依据。

二、创新性特色

1. 提出“kiss and hop”动态相互作用模型：

文章强调 tau 与微管的相互作用是高度动态的，而非传统认为的稳定结合，这解释了为何过度稳定 tau 反而有害。

2. 构建新型细胞模型用于药物筛选：

作者开发了一种结合 tau 突变体（如 TauΔK280）表达与活细胞成像技术的神经元模型，可同时评估 tau 聚集程度和其与微管的相互作用，为筛选双重作用药物提供了平台。

3. 提出“多药理策略”治疗理念：

文章主张理想的 tau 靶向药物应具备多重功能：抑制异常磷酸化、阻止新聚集形成、不溶解已有缠结、维持 tau 与微管的动态平衡。这种“多靶点”思路是对传统单一靶点策略的突破。

4. 对现有治疗策略的批判性反思：

文章指出：

- 降低总 tau 水平可能带来认知与情绪副作用；
- 溶解已形成的 tau 缠结可能释放有毒的可溶性 tau 寡聚体；
- 靶向 tau 微管结合区域的药物可能影响其同源蛋白 MAP2。

三、对学科的启示

1. 重新审视 tau 的生物学功能：

文章挑战了“tau 主要功能是稳定微管”的传统观点，强调其在轴突运输、信号传导等方面的多重角色，推动了对 tau 功能的再认识。

2. 推动疾病模型的发展：

作者指出当前模型（如鼠源神经元、过表达人 tau）的局限性，呼吁使用人源 iPSC 分化的神经元及更接近衰老环境的模型，以更好地模拟人类 tau 病变。

3. 强调治疗策略的“机制导向”：

文章倡导从“症状缓解”转向“机制干预”，强调在药物开发中必须综合考虑 tau 的生理与病理功能，避免“治病反致病”。

4. 为联合治疗提供理论支持：

文章提出的“多药理策略”为未来开发联合用药或多功能分子提供了方向，可能成为神经退行性疾病治疗的新趋势。

总结

这篇文章不仅在科学上深化了对 tau 蛋白功能与病理机制的理解，更在方法学上提出了创新的药物筛选模型与治疗理念，对推动神经退行性疾病从“对症治疗”向“机制治疗”转型具有重要的学术与临床意义。