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Mini Review

Cell-based Models To Investigate Tau Aggregation

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ABSTRACT

Accumulation of abnormal tau aggregates in neuron is an important pathological signature in multiple neurodegenerative disorders including Alzheimer's disease. Tau is a neuron specific microtubule-associated protein that regulates microtubule stability, which is critical for axonal outgrowth and synaptic plasticity. In a pathological condition, tau dissociates from microtubules and forms insoluble aggregates called neurofibrillary tangles (NFTs). The accumulation of NFTs in neuron directly correlates with microtubule dysfunction and neuronal degeneration. Due to the pathophysiological importance of tau, great efforts have been made to understand tau aggregation processes and find therapeutics to halt or reverse the processes. However, progress has been slow due to the lack of a suitable method for monitoring tau aggregation. In this mini-review, we will review the conventional methods for studying tau aggregation, and introduce recent cell-based sensor approaches that allow monitoring tau aggregation in living cells.

Contents

1. Introduction

Tau is a neuron specific microtubule-associated protein [1–3]. In a healthy neuron, tau binds to microtubules and regulates microtubule stability, which is critical for axonal outgrowth and neuronal plasticity [4–6]. When pathologically altered, tau molecules are not able to stabilize microtubules and become insoluble aggregates [3,7–9]. Since Alois Alzheimer discovered the abnormal tau inclusions in a patient's brain, the presence of tau aggregates is a critical biomarker for making the pathological diagnosis of AD [10]. In AD patients, three forms of tau aggregates occur; neurofibrillary tangles (NFTs) in neuronal somata, neuropil threads (NTs) in neuronal dendrites, and neuritic plaques (NPs). These tau aggregates induce neuronal degeneration. Especially, the density of NFTs correlates fairly well with regional and global aspects of cognitive decline during the progression of AD [10]. Hence, there has been great effort to understand how the deposition of NFT causes neurodegeneration (Fig. 1). NFT may damage neurons and glial

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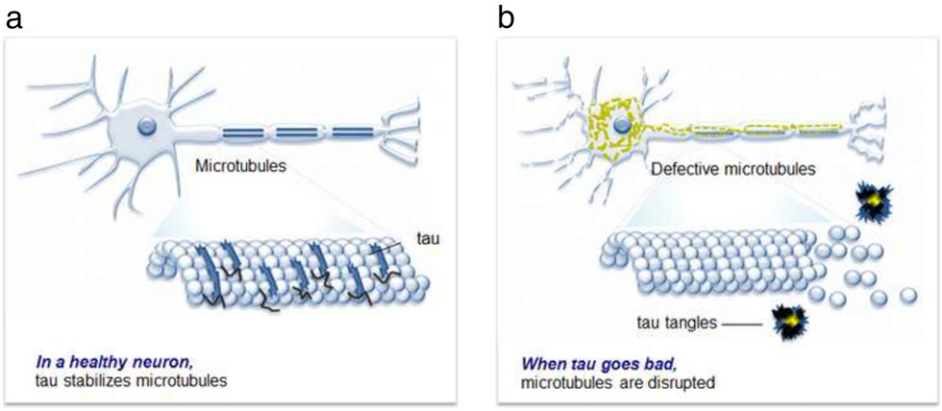

Fig. 1. Tau aggregation and neuronal degeneration [18]. (a) In a healthy neuron, tau stabilizes microtubules promoting axonal outgrowth and synaptic vesicle transport. (b) When tau goes bad, tau becomes neurotoxic aggregates and microtubules become dissociates.

cells in a number of ways [11]. First, aggregation of tau causes neuronal toxicity by reducing normal function of tau promoting microtubule stability. Also, the large filamentary tangles might be toxic to neurons by acting as physical barriers in the cytoplasm. Therefore, neurons containing tau tangles actively activate diverse cell metabolisms to get rid of the abnormal protein aggregates from cytoplasm [14,15]. This might be a great burden to a neuron that results in neuronal toxicity and neurodegeneration.

More recent studies suggest that, instead of the large insoluble filaments, soluble tau aggregates might play more critical roles in the onset and progression of disease prior to the development of NFT-induced neurotoxicity [6]. Especially hyperphosphorylated tau before NFT formation leads to microtubule disassembly, impairment of axonal transport, and organelle dysfunctions in neurons, leading to the neuronal cell apoptosis [3]. Also, the oligomeric species of tau may act as seeds for the aggregation of native tau, thereby promoting neurotoxic tau aggregation [8,16]. Accumulating evidence has suggested that tau aggregates are transmittable in neurons propagating as a prion-like manner [7,17].

Due to the pathological significance, tau becomes an important therapeutic target. Preventing tau aggregation becomes a potential strategy to cure neurodegenerative disorders associated with tau. So far, great effort has been made to identify molecular mechanism of tau aggregation and to reverse the processes. However, progress has been slow due to the lack of understanding the tau aggregation mechanism. Development of a reliable model for tau aggregation would be beneficial not only for identifying new therapeutic biomarkers but also for screening and evaluating drug candidates. Toward that, diverse tau aggregation methods have been developed: *in vitro*, cell-based, and *in vivo* models. Among the diverse models here we will review the *in vitro* and cell-based models for tau aggregation. *In vitro* tau aggregation methods have long been used for elucidating structural assembly of tau in the formation of PHFs. Cell-based models have recently developed to investigate the intracellular tau aggregation mechanism.

2. Multi-step Processes of Tau Aggregation

Contradictory to its pathological aggregation, tau is a naturally 'unfolded' protein, which is highly soluble in physiological condition [8,9]. To be a susceptible intermediate for aggregation tau molecules undergo a series of post-translational modifications and conformational changes in a neuron [19]. It is generally believed that tau aggregation is initiated by hyperphosphorylation (Fig. 2). Microtubule binding domains of tau contain a number of lysine residues, of which positive charges drive tau to bind negatively charged microtubules [20]. When tau is abnormally hyperphosphorylated, the balance is disrupted and tau dissociates from microtubules. Then, unbound tau undergoes conformational change to form a compact structure, called "Alz50 state" [21]. In this state, tau begins to aggregate and the further fibrillization is facilitated with proteolytic cleavages [1,13,22]. The truncated tau, named tau-66, assembles much faster than its native form [23]. NFTs are predominantly composed of paired helical filaments which appear to be made up of 10-nm filaments helically twisted with each other [24].

2.1. Tau Aggregation Assays In Vitro

To identify the ultra-molecular structure of PHFs, it is of obvious interest to reconstitute tau assembly process *in vitro* [12–14]. However, recombinant tau, which is purified from *Escherichia coli*, shows very little intrinsic tendency to aggregate *in vitro* due to the lack of a series of post-translational modifications required for aggregation. Over the last thirty years, the slow aggregation rate of purified tau has been improved by a combination of diverse advances (Table 1). First, recombinant truncated tau isoforms (*e.g.*, the repeat domain alone aggregates) are more frequently used for *in vitro* tau aggregation, instead of full-length tau. Tau-repeat domains such as K18 or K19 alone aggregate much faster than the full-length tau (Fig. 3) [25,26]. Second, tau mutations such as P301L or ΔK280 occurring in FTDP-17 are known to enhance the β-sheet propensity to increase the aggregation reaction [27–31]. Third,

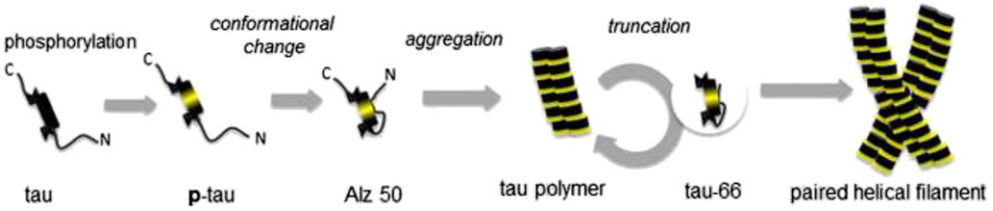

Fig. 2. Diagrammatic representation of tau aggregation [25].