# Chapter 2
# Toxic Tau Aggregation in AD

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The pathological hallmarks of Alzheimer's disease (AD) are extracellular β-amyloid deposition and intracellular tau inclusions. While β-amyloid deposition does not correlate with clinical progression of AD, the diffusion of neurofibrillary tangles (NFTs) from the entorhinal cortex to the neocortex, followed by neuronal and synapse loss, matches well with the clinical progression of AD symptomatology. Therefore, blocking the formation of NFTs is considered to be a promising approach to halt the progression of AD dementia.

Our analysis of the temporal formation of Tau fibrils in vitro showed that there are different and distinct forms of tau aggregates (soluble tau oligomer, and granular tau oligomer) that precede Tau fibril formation.

Analysis of our P301L-Tau Tg mouse model suggested that toxicity of Tau aggregates could be attributed to granular tau. To test this further, we attempted to reduce formation of granular tau oligomer by screening the chemical compound X1 that was shown to inhibit the formation of granular tau. Interestingly, oral administration of X1 to P301L-Tau mice resulted in reduced neuronal loss, accompanied by a reduction of the levels of sarkosyl-insoluble tau, as compared to control vehicle treatment. Together, these studies offer novel insights into Tau aggregation pathology; they strongly suggest that granular tau oligomers represent a toxic tau aggregate and that X1 may be a promising compound for blocking AD progression.

## 2.1 Introduction

In Alzheimer's disease (AD), β-amyloid deposition and neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau fibrils, are considered to be a major pathological feature. Evidence from the genetic studies of familial AD has given rise to the β-amyloid hypothesis, in which β-amyloid is proposed as the cause

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of AD; thus, the reduction of β-amyloid has been viewed as a potential therapeutic target for AD [1]. However, most studies targeting β-amyloid have failed in phase III clinical trial, as discussed extensively elsewhere [2]. A major explanation for such failures may be that such therapies have been tested in patients with early- to mid-stage AD when disease progression is already relatively advanced. In fact, when first AD symptoms are reported or detected, it seems that damage is irreversible and not amenable to slowing down or blockade. Accordingly, researchers are attempting to identify very early stages of disease, before conversion to, that may be therapeutically targetable; that is, they are now seeking an early "window of therapeutic opportunity." Even so, this approach depends on differentiating between the trigger and bullet in AD, and their relationship to β-amyloid. At the same time, researchers are becoming aware that alternative target for halting clinical progression even in early to moderate AD may be necessary.

## 2.2 Cause of Clinical Progression in AD

Tacitly, β-amyloid deposition may be the consequence, rather than the cause of disease. Indeed, all causative genes in familial AD relate to β-amyloid production, and the A673T protective APP mutation, which reduces Ab production [3]; A673T carriers show better cognitive scores than noncarriers. However, these findings suggest that not only β-amyloid is a cause of cognitive dysfunction, but also that dysfunction of APP or the products of other causatively linked genes are likely to be involved in β-amyloid production and cognitive dysfunction. Although β-amyloid toxicity has been observed in cultured neurons, mutant APP overexpressing mice do not show neuronal loss, but rather show memory impairment and senile plaques. Interestingly, immunotherapy against β-amyloid reduces β-amyloid deposition, and improves memory dysfunction in mutant APP overexpressing mouse model; however, immunotherapy does not halt clinical progression in AD patients, although immunotherapy reduces β-amyloid deposition in AD patients. Taken together, these observations suggest that β-amyloid is involved in Ab deposition and neuronal dysfunction, but not directly in neuronal death in the mature brain, explaining why β-amyloid targeting therapy does not prevent clinical progression of AD. It would appear that neuronal death may be a critical factor in clinical progression of the disease, indicating the need for alternative therapeutic targets and approaches.

## 2.3 Relationship Between NFTs and Clinical Progression of AD

NFTs are the other pathological hallmark of AD. While β-amyloid deposition is only seen in AD, NFTs are associated with several neurodegenerative diseases, the so-called tauopathies. Because all tauopathies are accompanied by NFTs and

neuronal dysfunction, NFTs are considered to be a common pathological marker for a range of neurological disorders. In these diseases, the rate of neuronal loss exceeds the occurrence of NFTs, suggesting that NFT formation and neuron death share a common underlying mechanism [4, 5]. This hypothesis is strongly supported by the discovery of a tau gene mutation in patients with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) [6–8]. The FTDP-17-associated tau gene mutation is the causal factor in FTDP-17, a dementing disease characterized by NFT formation and neuron loss. Analyses of mutated tau in FTDP-17 conclusively demonstrated that tau dysfunction or abnormality alone induces the neurodegeneration characterized by NFTs and neuronal death that ultimately leads to clinical dementia.

## 2.4 NFTs and Neuronal Dysfunction

Braak and colleagues defined the progression of AD in terms of six stages, based on the distribution of NFTs in the brain [9]. In Braak stage I, NFTs are observed in the transentorhinal cortex and the CA1 region of the hippocampus. The number of NFTs increases in Braak stage II, and Braak stages I and II together are called the transentorhinal stage. Brains from normal non-demented aged subjects are also often categorized as Braak stages I and II. In Braak stages III and IV, called the limbic stage, many ghost tangles appear in the entorhinal cortex, and NFTs are found throughout the entire limbic system, including hippocampal regions CA1-4 and the amygdala. In the limbic stage, patients show various AD-specific symptoms, such as memory impairment, reduced spatial cognition, and reduced motivation, ascribable to neural dysfunction in the limbic system. In Braak stages V and VI, called the isocortical stage, NFTs are present in the cerebral cortex, where they impair neural function and cause dementia. The increasing spread of NFTs from the transentorhinal cortex to the limbic system and finally to the cerebral cortex correlates with increasing impairment of brain function. Samuel and colleagues reported that the number of NFTs in CA1, subiculum, and CA4 of the hippocampal formation correlates with the degree of dementia [10]. Therefore, the distribution of NFTs is now considered to correlate better with disease progression in AD than β-amyloid production and deposition.

## 2.5 Intermediate Form of NFT

Mice that overexpress P301L mutant tau under the regulation of a tetracycline-inducible promoter display age-related NFTs, neuronal death, and behavioral deficits. Although inhibition of mutant tau in these mice blocks neuronal death and improves memory, NFTs continue to form [11–13]. This suggests that NFTs are

not themselves toxic, but rather, that the mechanism of NFT formation is shared by the process underlying neuronal death and neuronal dysfunction.

To understand a process of NFT formation that links with neuronal death, we first need to know how monomeric tau forms fibrillar tau. Anionic surfactants accelerate fibril formation of tau protein in vitro, and fibril formation can then be monitored by thioflavin (ThT) fluorescence, which recognizes protein aggregations with a β-sheet conformation. To track structural changes in tau in solution, and to understand the relationship between different tau aggregates, Maeda and colleagues investigated how tau assembly in vitro changes over time by measuring ThT fluorescence, using atomic force microscopy (AFM) [14].

Before ThT fluorescence can be detected, tau forms oligomers (dimers to octamers) with increasing time of incubation [15]. Although these tau oligomers could not be observed under AFM, they were detected by SDS-PAGE performed under nonreducing conditions. Under reducing conditions, however, tau oligomers, consisting of three or more tau molecules, could no longer be detected, suggesting that tau oligomers form through disulfide bonds and through other SDS-resistant tau–tau associations. As ThT fluorescence increases, two forms of tau aggregates can be observed with AFM: a granular tau oligomer and a fibrillar tau aggregate. Laser light-scattering analysis of sucrose-gradient purified granular tau oligomers indicated that a single granular tau oligomer consists of about 40 tau molecules. Since higher concentrations of granular tau oligomer induce the formation of tau fibrils, it has been proposed that granular tau oligomer is an intermediate form of tau fibrils [14]. Thus, monomeric tau molecules first bind to each other through disulfide bonds and SDS-resistant interactions to form tau oligomers that are not visible under AFM. Forty tau molecules bind together forming a β-sheet structure; these 40-tau aggregates, which appear granular in shape under AFM, accumulate to form tau fibrils.

## 2.6 Role of Granular Tau Oligomers in Neurodegeneration

Before forming fibrillar aggregates, tau forms two different types of tau aggregate: oligomeric tau (sarkosyl-soluble, not detectable by AFM) and granular oligomeric tau (sarkosyl-insoluble, detectable under AFM). If NFTs represent tombstones of neurodegeneration, these two kinds of intermediate tau oligomer may play a role in synapse loss, neuron loss, and ultimately, the neurodegeneration typically seen in tauopathies.

Kimura and colleagues [16] generated a transgenic (Tg) mouse line expressing P301L human tau. These mice display sarkosyl-insoluble tau aggregates and neuronal loss, but not typical NFTs. Because the pool of insoluble tau includes both fibrillar and granular forms of tau aggregates, and because NFTs are not a toxic species of tau [12–14], granular tau oligomers may be the form of tau involved in neuronal loss. This premise is in accord with the finding that the frontal cortex of

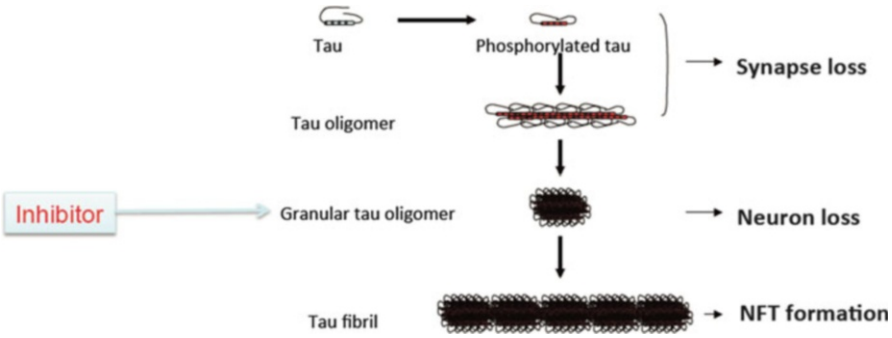


**Fig. 2.1** Tau-targeting therapy. Before NFT formation, tau aggregates, and form tau oligomer, and granular tau oligomer. Hyperphosphorylated tau and tau oligomers are involved in synapse loss, and Granular tau oligomer causes neuronal loss. Blocking granular tau oligomer formation results in inhibition of neuronal loss accompanying ameliorate neuronal dysfunction in mouse model

Braak stage I brains contains elevated levels of granular tau, because the frontal lobe is particularly susceptible to volume decreases with aging [17].

Figure 2.1 summarizes the processes involved in tauopathy-associated neurodegeneration. As shown, hyperphosphorylated tau dislodges from microtubules, and since it has a higher affinity for participating in tau–tau interactions, individual hyperphosphorylated tau molecules bind to each other to form oligomeric tau; the latter is detergent-soluble and cannot be detected under AFM. Hyperphosphorylated tau or oligomeric tau is involved in synaptic loss, as demonstrated in studies of wild-type human tau Tg mice [16]. When the tau oligomer grows to about 40 molecules, it takes on a β-sheet structure and becomes a detergent-insoluble aggregate, visualized as a granular structure under AFM. Unlike hyperphosphorylated tau or oligomeric tau, granular tau oligomers may be involved in neuronal loss. As granular tau oligomers fuse together, they form tau fibrils, which ultimately form NFTs. Thus, different forms of tau aggregates are involved in the different pathological changes that occur in tauopathies. The mechanism underlying the loss of synapses and neurons seen in tauopathies may be the same mechanism underlying the neuropathology seen in other neurodegenerative diseases characterized by intracellular protein inclusions.

## 2.7 Tau-Targeting Therapy

As P301L-Tau mice do not form tau fibrils but nevertheless exhibit neuronal loss, we suggest that granular Tau is responsible for the toxicity of Tau aggregates. To test this notion further, we attempted to reduce formation of granular tau oligomers with the compound X1 which has the ability to inhibit the formation of granular tau. Interestingly, oral administration of X1 to P301L-Tau mice reduces neuronal loss,

accompanied by a reduction of sarkosyl-insoluble tau levels, as compared to vehicle treatment. Taken together, our studies offer novel insights into Tau aggregation pathology; they strongly suggest that granular tau oligomers represent a toxic form of aggregated tau, and that X1 may be a promising compound for blocking the progression of AD.

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