Potential of the Antibody Against cis-Phosphorylated Tau in the Early Diagnosis, Treatment, and Prevention of Alzheimer Disease and Brain Injury

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Alzheimer disease (AD) and chronic traumatic encephalopathy (CTE) share a common neuropathologic signature—neurofibrillary tangles made of phosphorylated tau—but do not have the same pathogenesis or symptoms. Although whether traumatic brain injury (TBI) could cause AD has not been established, CTE is shown to be associated with TBI. Until recently, whether and how TBI leads to tau-mediated neurodegeneration was unknown.

The unique prolyl isomerase Pin1 protects against the development of tau-mediated neurodegeneration in AD by converting the phosphorylated Thr231-Pro motif in tau (ptau) from the pathogenic cis conformation to the physiologic trans conformation, thereby restoring ptau function. The recent development of antibodies able to distinguish and eliminate both conformations specifically has led to the discovery of cis-ptau as a precursor of tau-induced pathologic change and an early driver of neurodegeneration that directly links TBI to CTE and possibly to AD. Within hours of TBI in mice or neuronal stress in vitro, neurons prominently produce cis-ptau, which causes and spreads cis-ptau pathologic changes, termed cistauosis. Cistauosis eventually leads to widespread tau-mediated neurodegeneration and brain atrophy. Cistauosis is effectively blocked by the cis-ptau antibody, which targets intracellular cis-ptau for proteasome-mediated degradation and prevents extracellular cis-ptau from spreading to other neurons. Treating TBI mice with cis-ptau antibody not only blocks early cistauosis but also prevents development and spreading of tau-mediated neurodegeneration and brain atrophy and restores brain histopathologic features and functional outcomes. Thus, cistauosis is a common early disease mechanism for AD, TBI, and CTE, and cis-ptau and its antibody may be useful for early diagnosis, treatment, and prevention of these devastating diseases.
(ptau) from the pathogenic cis isomer to the physiologic trans isomer. The recent development of antibodies able to specifically distinguish and eliminate both conformations has led to the discovery that cis- ptau is an early pathogenic tau conformation that instigates and propagates neurodegeneration, which directly links TBI to CTE and offers a possible common molecular link among AD, TBI, and CTE.30,31

Within hours after neuronal stress in vitro or after rmTBI or ssTBI in mouse models, neurons prominently produce cis- ptau, which causes a pathogenic process in vitro and in vivo termed cistauosis.31 Cistauosis in these murine models eventually leads to widespread tau-mediated neurodegeneration and brain atrophy,33 both of which are common features of CTE and AD. Treatment with the cis- ptau antibody not only neutralizes cis- ptau and stops cistauosis but prevents secondary brain damage after TBI and halts the later development of widespread tau-mediated neurodegeneration and brain atrophy in these animal models.31 Thus, cis- ptau is an early driver of TBI and mediates the progression of TBI to CTE. Future studies are needed to confirm these results in independent settings and to determine how cis- ptau induces neurotoxicity and interferes with other pathogenic tau modifications, such as acetylation,33 during the development of tau-induced neurodegeneration. The cis- ptau antibody nevertheless offers a promising approach for early treatment of AD, TBI, and CTE. Furthermore, measurement of cis- ptau levels in bodily fluids could represent a means to diagnose these disorders in their early stages and possibly observe disease progression and treatment efficacy.

**Tau Tangles as a Neuropathologic Signature of AD and CTE**

Nefrofibrillary tangles are a neuropathologic hallmark of AD and other neurodegenerative disorders, together known as tauopathies.33,34 Recent studies of brains from boxers, US football players, and blast-exposed military veterans with CTE have identified extensive neurofibrillary tangles as a common neuropathologic signature, although they have different clinical trajectories and this subject is still hotly debated.2,17 Although the distribution and appearance of tau-mediated neurodegeneration in the brains of patients with AD and CTE are not the same,5,6,33,34 the tau isoform profile and phosphorylation state of tangles purified from brains with AD and brains of boxers with CTE are indistinguishable,35 suggesting a potentially similar pathologic mechanism. However, because few pathogenic tau epitopes are detectable acutely or subacutely after TBI in humans and mice,3,5,7,36-38 whether tau-induced pathologic changes represent the end result of degenerative pathologic change or an early driver of brain injury is unclear.

Tau protein has an important biological function in stabilizing the axonal microtubule network in neurons critical for normal brain function.33,34 Loss of tau function in neurodegenerative diseases is well established, but how tau becomes pathogenic and leads to dementia remains unclear.33,34 Tau hyperphosphorylation, especially on Ser or Thr residues preceding a Pro residue (pSer/Thr-Pro), is an early event preceding tangle formation in AD.39 Such phosphorylation has been shown to disrupt microtubule function, alter protein stability, cause tau oligomerization and aggregation, and eventually lead to tangle formation.33,34 The identification of tau mutations in patients with frontotemporal dementia and parkinsonism linked to chromosome 17 has established tau as a causative factor in neurodegeneration.40-42 Mice that overexpress tau, especially tau mutants or normal human tau without mouse tau, develop tangles.43 Moreover, tau kinases44 or phosphatases45,46 are deregulated in AD, and modulating them can affect tangle formation in mice. Finally, tau-mediated neurodegeneration can spread in the brain,47-51 but active and passive immunization against pathologic tau epitopes,52,53 including tau seeding54 or tau oligomers,55 reduces tau aggregates and improves memory deficits in mouse models. How phosphorylation can turn tau, which serves a vital physiologic function in healthy neurons, into a pathogenic agent leading to dementia in the setting of TBI and/or AD is not fully understood. We still need to elucidate whether tau is further regulated after phosphorylation before we can develop appropriate therapeutics to block pathogenic ptau without detrimental effects to physiologic tau.

**Pin1 and Conversion of pTau From Cis to Trans Isomers**

Proline-directed Ser/Thr phosphorylation is a central common signaling mechanism in the cell.56 Investigators21,57,58 have previously identified a unique prolyl isomerase, Pin1, that catalyzes cis-trans isomerization of certain pSer/Thr-Pro motifs in a phosphorylation-dependent manner. Pin1 is tightly regulated but is inhibited during aging by many different mechanisms.7,22 Extensive in vitro and in vivo research has shown that Pin1 binds to ptau and inhibits the development of tau-mediated neurodegeneration in AD by catalyzing the cis to trans conversion of ptau.26,59 Pin1-catalyzed conformational changes (1) restore the ability of ptau to promote microtubule assembly22; (2) facilitate ptau dephosphorylation, which cannot be performed by the trans-specific protein phosphatase 2A23,60; and (3) promote ptau degradation24 (Figure 1). Pin1 has no effect on the Thr231A mutant tau,22,24,60 although Pin1 can bind or isomerize to other motifs in vitro.60,62 In vitro studies showed that Pin1 did not catalyze isomerization of the pThr231-Pro motif63 and did not regulate the microtubule function of ptau.64 However, these conclusions are mainly based on their findings that Pin1-catalyzed cis-trans isomerization of pThr231-Pro ptau peptides was not faster than the detection limit (0.1 millisecond) of the nuclear magnetic resonance spectroscopic NMR technique used63 and that Pin1 did not promote ptau dephosphorylation.64 Moreover, these studies are not validated by any evidence from in vivo studies showing the physiologic relevance of the findings.63,64 In fact, the ability of Pin1 to isomerize ptau and promote ptau dephosphorylation and microtubule functions has been documented by multiple groups in vitro, in neurons, and in mice, even using cis- and trans- ptau polyclonal and monoclonal antibodies (mAbs).22,24,60,65

In support of Pin1’s role in the pathophysiological features of AD, Pin1 knockout mice are the only murine models that display both tau-related and β amyloid (Aβ)-related depositions and neurodegeneration in an age-dependent manner,3,25 whereas Pin1 overexpression prevents tau aggregation and neurodegeneration in mice overexpressing wild-type human tau.24 In human AD studies,22,23,26,29 Pin1 is specifically inhibited in neurons by various
Cistauosis as an Early Druggable Mechanism in Alzheimer Disease (AD), Traumatic Brain Injury (TBI), and Chronic Traumatic Encephalopathy (CTE)

When tau is phosphorylated on the specific motif Thr231-Pro (ptau), it exists in 2 distinct conformations. The trans conformation promotes microtubule assembly critical for normal neuron function, whereas the cis conformation causes cistauosis, which includes the disruption of the axonal microtubule network and mitochondrial transport, spreading to other neurons, and leading to neuron death by apoptosis. The unique isomerase Pin1 protects against the development of tau-induced disease by accelerating cis to trans isomerization to prevent the accumulation of the pathogenic cis- ptau, notably in the axons of neurons. However, in AD, TBI, and CTE, Pin1 function is inhibited and cis- ptau is accumulated prominently in diffuse axons, thereby causing cistauosis and spreading axonal disruption with time, eventually leading to tau-mediated neurodegeneration and brain atrophy. Cistauosis and cis- ptau can be effectively and specifically neutralized by the cis- ptau monoclonal antibody (mAb), which also potently stops brain damage after TBI and prevents the late development of neurodegeneration, such as CTE, in animal models. LTP indicates long-term potentiation; P, Pro; and pT, phosphorylated Thr231.

Cis-pTau as an Early Pathogenic Conformation Leading to Tau-Mediated Neurodegeneration in AD

The recent development of polyclonal antibodies capable of distinguishing cis- ptau from trans- ptau using innovative peptide chemistries to increase the cis content in antigenic peptides30 has provided a tool to distinguish between cis- tau and trans- tau conformations. These antibodies have shown that cis- ptau but not trans- ptau appears in human brains with early mild cognitive impairment. Cis- ptau accumulates exclusively in degenerated neurons, localizes to dystrophic neurites as AD progresses, and correlates well with cognitive deficits30 (Figure 1). Furthermore, in contrast to trans- ptau, which promotes microtubule assembly, the cis isomer loses normal function and gains toxic function, becoming more resistant to dephosphorylation and degradation and more prone to protein aggregation (Figure 1). Pin1 converts cis- ptau to trans- ptau to prevent tau-mediated neurodegeneration in AD.30 These results indicate that cis- ptau but not trans- ptau is an early pathogenic tau conformation that can lead to the development of tau-mediated neurodegeneration in AD and suggest that conformation-specific antibodies and vaccines could be developed for the early diagnosis and treatment of Alzheimer disease30 (Figure 1).

Cistauosis as an Early Precursor of Tau-Mediated Neurodegeneration Linking TBI to CTE

To test whether cistauosis is an early precursor of tau-mediated neurodegeneration that links TBI and CTE, highly specific and potent cis- ptau and trans- ptau mAbs without any cross-reactivity were generated using peptide chemistry that allows the generation of cis and trans polyclonal antibodies.30,31 Using conformation-specific mAbs, these studies showed that, although trans- ptau is detected in a very few neurons in the soma even in normal brains, no cis- ptau is detectable in normal brains. However, robust cis- ptau but not trans- ptau is notable with prominent localization to diffuse axons in sport-
and military-related human brains with CTE and their respective TBI mouse models.31

To demonstrate the significance of cis-ptau induction, Kondo et al31 exposed cultured neurons to hypoxia or serum starvation, similar to TBI. Both conditions strongly induced cis-ptau, causing cistauosis, including the disruption of the axonal microtubule network and mitochondrial transport, which spread to other neurons and result in eventual massive apoptosis. Cistauosis is enhanced by the trans-mAb but fully blocked by the cis-ptau mAb, which enters neurons via Fcγ receptors to target intracellular cis-ptau for protein degradation by the tripartite motif-containing 21–mediated proteasome pathway and to prevent extracellular cis-ptau from spreading to other neurons31 (Figure 2). In addition, tau neurotoxicity is potently induced by cotransfection with its kinase p25-activated cyclin-dependent kinase 5, which is fully suppressed by the Thr231Ala tau mutation, total tau mAb, or cis-mAb but enhanced by trans-mAbs.33 These results are highly significant because p25 overexpression results in tau phosphorylation and massive neuronal death in mouse brains.73 Because cyclin-dependent kinase 5 p25 phosphorylates tau on many sites, and the Thr231Ala mutation does not affect other tau phosphorylation sites,22 these results indicate that cis-pT231-tau is necessary and sufficient for ptau to induce neurotoxicity in vitro conditions.31

The significance of cis-ptau in TBI is further demonstrated in mouse models mimicking sport (impact)– and military (blast)–related closed head injury.31 Diffuse axonal injury has emerged as one of the most common and important pathologic features of closed head injury, the most common form of TBI.74 Diffuse axonal injury is conventionally recognized to cause disruption in axonal transport, followed by secondary disconnection and finally wallerian degeneration.74,75 Although this process was traditionally thought to be limited to the acute and subacute periods, recent evidence has identified axonal degeneration in human brains years after injury.74,76 Notably, this axonal degeneration may have a role in the development of AD-like disease in the acute and chronic phases after TBI.74,75,77,78 However, molecules that mediate from diffuse axonal injury to axonal degeneration remain elusive.74 Recent results suggest that cis-ptau is a strong candidate for these elusive mediators because cis-ptau causes and spreads axonal degeneration in the acute and chronic phases after TBI, as described below. Traumatic brain injury induces cis-ptau in a dose-dependent manner.31 Whereas mild TBI causes transient and modest cis-ptau induction, rmTBI or ssTBI can cause persistent and robust cis-ptau induction within 12 to 24 hours after injury, long before any other known tau pathogenic changes can be detected, including tau oligomerization, aggregation, and tangle formation.31 Cis-ptau diffusely localizes to axons, disrupts the axonal microtubule network and mitochondrial transport, induces apoptosis, and eventually spreads through the brain over time. These changes result in the appearance and spreading of cistauosis phenotypes in the brain, which closely resemble those in cultured neurons31 (Figure 2). Moreover, as with in vitro experiments, cis-ptau mAb treatment after ssTBI effectively blocks induction of cis-tau and cistauosis in murine brains and fully prevents the development and spread of TBI-related tau pathologic changes, deposition, defective long-term potentiation and anxiety or risk-taking behavior, and brain atrophy31 (Figure 2). The kinetics of cis-ptau induction and its neurotoxicity under various TBI conditions are consistent with clinical observations that very mild TBI may have limited long-term sequelae, whereas rmTBI and ssTBI can be associated with acute neurologic dysfunction, long-term cognitive disability, and a pathologic finding of CTE.2–7 Given the major role of cis-ptau in AD,22–24,30 together with previous epidemiologic observations that TBI might increase the risk for dementia,12,17 the above results indicate that cistauosis might be a common early disease mechanism not only in AD but in TBI and CTE,31 thus offering a potential mechanistic link between TBI and CTE. However, further experiments are needed to investigate molecular details of how cistauosis causes neurodegeneration in CTE and whether and how TBI might cause Alzheimer disease remains to be determined. Arrows indicate the likelihood of developing CTE.

Figure 2. A Model for Immunotherapy Targeting the Early Disease Driver Cis-Tau Phosphorylated on the Specific Motif Thr231-Pro (pTau)

Traumatic brain injury (TBI) induces cis-ptau in a dose-dependent manner. Whereas a single concussion (mild TBI) causes transient and modest cis-ptau induction that returns to the baseline within a couple of weeks, repetitive mild TBI or concussion, as seen in contact sports, or single moderate or severe TBI, as seen in exposure to blasts in the military or in road traffic crashes, causes persistent and robust cis-ptau induction, notably in diffuse axons within 12 to 24 hours after injury, long before any other known tau pathogenic changes. Cis-ptau causes cistauosis and spreads axonal disruption in the acute and chronic phases of TBI, which affects brain function and, years later, leads to widespread tau-mediated neurodegeneration and brain atrophy, a common feature of chronic traumatic encephalopathy (CTE), which are effectively blocked by treating TBI in mice with cis-ptau monoclonal antibody (mAb).

Based on these results, we speculate that cis-ptau mAb may be used to develop diagnostic tests that could detect harmful levels of cis-ptau soon after TBI and also to develop a therapeutic antibody to halt brain damage after TBI, which would prevent the later development of progressive neurodegeneration, such as CTE. Whether and how TBI might cause Alzheimer disease remains to be determined. Arrows indicate the likelihood of developing CTE.

Cis-pTau-Based Early Diagnosis and Treatment of AD, TBI, and CTE

Immunotherapies remain the largest class of human drugs because they are highly effective and specific in attacking their intended targets, and vaccines can be administered long before disease develops and have minimal adverse effects.79 Immunotherapy using Aβ vaccines or peripherally administered humanized Aβ mAbs can reduce brain Aβ levels and clear Aβ plaques in mouse models of AD.80–83 This outcome was also seen in patients with AD, even in phase 3 clinical trials, but memory loss was not improved.84–87
Tau has become an attractive drug target for a number of reasons. Unlike senile plaques, tau tangles correlate well with neuronal loss and cognitive decline in patients with AD. Furthermore, tau tangles are a defining feature of many human tauopathies without Aβ plaques, including CTE. Active and passive immunization against tau tangle epitopes as well as tau seeding can reduce tau aggregates and improve memory deficits in mouse models, along with tau clearance from the brain to the blood. In addition, antibodies directed against ptau, cis-Aβ40, or α-synuclein can be internalized to clear their respective intraneuronal aggregates. However, because commonly known antibodies targeting pathogenic tau often appear months after TBI in mice and years after TBI in humans, immunotherapy must selectively target the earliest possible pathogenic tau without affecting functional tau in AD and TBI.

Recent results offer a promising new therapy for stopping secondary brain damage after TBI and preventing its long-term sequelae (Figure 2). For example, ptau in cerebrospinal fluid is a well-known AD biomarker useful for diagnosing and tracking AD progression. However, the levels of ptau in the cerebrospinal fluid in AD show huge individual variation. The discovery that ptau exists in the diseasing-causing cis and physiologic trans conformation in AD and TBI opens the possibility of using conformation-specific antibodies to identify appropriate patients with TBI for cis- ptau–targeted therapy and to assess its therapeutic response (Figure 2). Moreover, given the potency of cis-mAb in stopping cistauosis and brain damage after TBI and preventing its neurodegeneration, as shown in murine models, cis-ptau antibodies and even vaccines might be further developed to treat or prevent not only AD but also TBI and CTE (Figure 2).

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