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Title Page

Hyperphosphorylated Tau is Implicated in Acquired Epilepsy and Neuropsychiatric Comorbidities

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Abstract Epilepsy is a common neurological disease. Acquired epilepsy can be caused by brain insults, such as trauma, infection, or tumor, and followed by a latent period from several months to years before the emergence of recurrent spontaneous seizures. More than 50% of epilepsy cases will develop chronic neurodegenerative, neurocognitive and neuropsychiatric comorbidities. It is important to understand the mechanisms by which a brain insult results in acquired epilepsy and comorbidities in order to identify targets for novel therapeutic interventions that may mitigate these outcomes. Recent studies have implicated the hyperphosphorylated tubulin-associated protein (tau) in rodent models of epilepsy and Alzheimer's disease, and in experimental and clinical studies of traumatic brain injury. This potentially represents a novel target to mitigate epilepsy, and associated neurocognitive and psychiatric disorders post brain injury. This article reviews the potential role of tau based mechanisms in the pathophysiology of acquired epilepsy, and its neurocognitive and neuropsychiatric comorbidities, and the potential to target these for novel disease-modifying treatments.

Keywords Hyperphosphorylated tau; Acquired epilepsy; Traumatic brain injury; Post-status epilepticus; Neuropsychiatric comorbidities

Introduction

The Problem of Acquired Epilepsy

Epilepsy is one of the most common acquired chronic neurological diseases affecting approximately 50 million people worldwide [1]. It is characterized by the occurrence of recurrent unprovoked seizures. Some epilepsy cases are idiopathic, in that there are no identified causes for the epilepsy. In contrast, other cases develop following a brain insult (trauma, status epilepticus), and are referred to as "acquired epilepsy". These epilepsies often develop after a latent period between the initial insult and the onset of the seizures of at least 3 months and sometimes up to 20 years, [2]. Patients with more severe brain injuries have a higher incidence of epilepsy. [3]. Although great advances have been achieved in the development of modern antiepileptic drugs (AEDs) and progress of surgical treatment, up to 50% of acquired epilepsy cases remain treatment resistant [4].

Neurodegeneration and Neuropsychiatric Comorbidities of Acquired Epilepsy

More than 50% of patients with acquired epilepsy have associated neuropsychiatric (psychosis and depression) [5] and cognitive (memory, attention, and executive speed) [6] comorbidities, which have important negative impacts on the patients quality of life. To date, there are no specific pharmaceutical interventions targeting these comorbidities in patients with epilepsy [7]. A recent study from our group found a relationship between abnormal brain structures and psychiatric comorbidity in Genetic Absence Epilepsy Rats from Strasbourg (GAERS), an animal model of genetic generalized epilepsy with absence seizures that manifests increased anxiety and depressive behaviours [8]. The volumes of the amygdala, cortex and ventricles were increased in these rats compared to non-epileptic control rats (NEC). Furthermore, an abnormal shape of the hippocampus in the GAERS was found. This study suggests that limbic structures including the hippocampus and amygdala might be involved in the development of neurobehavioural comorbidities of epilepsy [8]. A recent study showed development of atrophy and hypometabolism in the limbic structures in a post-status epilepticus model of acquired epilepsy [9]. The limbic system is involved in a variety of neuropsychiatric diseases, such as emotional and behavioural disorders, stress dysregulation, motivation and long-term memory loss. Structural and functional changes in the hippocampus and the amygdala, may be implicated in the pathological

mechanism of neurobehavioural disorders in epilepsy [8,9]. Accordingly, memory deficit is the most common complaint in patients with mesial temporal lobe epilepsy (MTLE) [10].

The Need for Anti-Epileptogenic and Neuroprotective Treatments for Acquired Epilepsy

All current AEDs only suppress seizures, with none confirmed to stop or reverse the process which converts a healthy brain into an epileptic brain – a process known as epileptogenesis [11]. One of the major goals of translational research in the epilepsy field is to develop disease-modifying treatments that inhibit or reverse epileptogenesis [12]. Understanding the mechanisms that link acquired epilepsy, neurobehavioural disorders and neurodegeneration may provide important insights into the cellular and molecular basis of the disease. As there is a latent period following the causative insult and the development of the epilepsy, this provides a time window of opportunity for pharmaceutical treatment to intervene in the occurrence of epilepsy and comorbidities. The challenge lies in identifying such protective compounds and confirming their efficacy and safety.

Traumatic Brain Injury as a Cause of Acquired Epilepsy

Traumatic brain injury (TBI) is an important cause of death and disability in adults in developed and developing countries [13], and its incidence rate is rising worldwide as a result of motor vehicles, sporting activities and wars. Despite the high prevalence of TBI, there is a poor understanding of the pathophysiological mechanisms involved with the injury. No effective pharmaceutical interventions are available to reduce the adverse long term consequences, such as cognitive dysfunction, emotional abnormalities and motor disabilities. Approximately 25% of patients with severe TBI will develop post-traumatic epilepsy [14] and TBI has been associated with several neurodegenerative conditions such as chronic traumatic encephalopathy and Alzheimer's disease [15]. Taken together, more than 50% of moderate and severe TBI patients endure long-term TBI-related disabilities with poor outcome [16]. TBI often requires prolonged rehabilitation and care, which leads to a significant cost to patients, their relatives, and societies [17, 18].

Basic Pathophysiology of TBI

The pathophysiological processes in the brain associated with TBI can be categorized as resulting from either primary or secondary injuries. The primary injury is induced at the moment of impact by mechanical forces that result in the rapid shift or distortion of brain tissue, focal contusion, and diffuse injury in brain parenchyma and vascular structures [19]. Given the rapid onset of these injuries, they are largely considered irreversible. Conversely, common secondary injury processes such as excitotoxicity [20], oxidative stress [21], neuroinflammation [22], and other structural or chemical alterations [23], are often initiated by the primary insult but may take days to years to fully manifest. Given this delayed onset, it is possible that secondary injuries may be prevented and are considered to be potential pharmaceutical targets to improve TBI outcomes [24]. These secondary mechanisms represent complex, parallel, interacting and interdependent injury processes that remain poorly understood, with no pharmacological treatment known to effectively limit their progression and improve TBI patient outcome.

Hyperphosphorylated Tau in TBI

Hyperphosphorylated tau and related pathologies such as neurofibrillary tangles (NFTs), have received increased attention from the medical community in recent years in relation to the pathophysiology of a wide variety of neurodegenerative conditions. Originally identified as a critical pathology in Alzheimer's disease (AD), hyperphosphorylated tau has since been implicated in other neurodegenerative conditions including the tauopathies (frontotemporal dementia, progressive supranuclear palsy), Parkinson's disease (PD), epilepsy, and TBI. Accumulating evidence is implicating tau pathologies in the pathophysiological aftermath of TBI [25]. Widespread intra-axonal accumulations of total and hyperphosphorylated-tau have been demonstrated in the brains of patients with severe TBI [26]. TBI increases the risk of AD [27], PD [28], and post-traumatic epilepsy (PTE) [14], all conditions in which tau-based mechanisms may play important roles. Patients who have suffered repeated concussive brain injuries may develop a chronic traumatic encephalopathy with neuropathological neurodegenerative changes including increased tau and hyperphosphorylated tau [15].

Tau Based Mechanisms in Acquired Epilepsy

A number of lines of evidence suggest that common pathological mechanisms may be involved in the pathophysiology of acquired epilepsy and of associated neurodegenerative comorbidities [29, 30]. Patients with AD have an increased incidence rate of epilepsy compared with age-matched healthy controls [31]. A recent cohort study of 3,078 subjects with mild to moderate AD found that risk factors such as: younger age, cognitive dysfunction, and use of antipsychotic drug are related to the development of epilepsy [25, 32]. Neurofibrillary tangles composed of hyperphosphorylated tau have also been identified in surgical specimens from patients with focal cortical dysplasia and medically refractory epilepsy, and these hyperphosphorylated tau tangles are specifically localized to the dysplastic regions [15, 33].

Notably, excitotoxicity is a potential pathophysiological mechanism in TBI [20], AD [30], and acquired epilepsy [30], and promotes the abnormal hyperphosphorylation of tau. For example, the seizure-inducing excitotoxin kainic acid (KA) is widely used experimentally to induce a model of acquired epilepsy as most rodents will develop spontaneous recurrent seizures and neuropathological changes including atrophy of limbic structures after KA exposure [9]. Of relevance, KA has also been shown to decrease tau phosphorylation initially and then increase and sustain the hyperphosphorylation of tau in the hippocampus [34]. In this study, Liang et al found the decreased activity of PP2A was correlated with tau phosphorylation. Furthermore, although KA increased the activity of mitogen-activated protein kinase (MAPK) and glycogen synthase kinase 3 (GSK3)-beta, these changes were not associated with tau phosphorylation abnormalities [34].

Physiological and Pathological Functions of Tau

Under normal physiological conditions, tau is in a balance of binding or detaching with the microtubules (MTs). This balance is considered to be primarily regulated by partially phosphorylated tau [29], which in turn is controlled by a large number of kinases and phosphatases, and enables tau to stabilize MTs [35]. In contrast, under pathological conditions this equilibrium is perturbed and hyperphosporylated tau is markedly over-produced [36], resulting in destabilization of MTs.

The phosphorylation of tau in brain is regulated by various protein kinases, including cyclin-dependent kinase 5 (Cdk5) and GSK3 which target serine and threonine residues in proline-rich domains of the protein [37], and non-proline directed kinases such as protein kinase A (PKA) and the MT-affinity-regulating kinase (MARK) [38]. Many of these kinases have been explored as potential pharmaceutical targets by using inhibitory compounds [29]. In an in-vivo study of transgenic mice with AD, a GSK 3 beta inhibitor decreased the expression of phosphorylated tau [31], decreased the level of aggregated tau [39], arrested the development of NFT [31, 40], and reversed the neuronal injury [41]. The proposed mechanism for these observations was that GSK 3 beta mediated phosphorylation of tau at the Thr231 site induces the C-terminus of tau protein into a hyperphosphorylation state, which plays a critical role in regulating the affinity between tau and MTs [33]. However, other kinases, such as CDK5, phosphorylate the tau protein at the Thr231 site [42]. Lee et al [33] used AD transgenic mice model to confirm the function of CDK5 in tau phosphorylation, aggregation and formation of NFT. In addition, the mutant mice which overexpressed p25, a CDK5 activator, showed over-expression of hyperphosphorylated tau in the brain [43]. The specific role and relationship of the different kinases in specific diseases, such as TBI, epilepsy or AD, remain unclear, making them ambiguous pharmaceutical targets.

Tau phosphatases reverse the actions of these protein kinases, and have been implicated in tauopathies [44]. For example, the activities of protein phosphatases 20-30% lower in the brains of AD patients compared with age-matched controls [45]. A single phosphatase, PP2A (protein phosphatase 2A), accounts for over 70% of tau phosphatase activity in human brain [46]. Of interest, previous research investigating the function of PP2A in the formation of hyperphosphorylated tau reported that inhibition of PP2A activity by okadaic acid (OA) in vitro and in vivo, resulted in abnormal hyperphosphorylation of tau at several sites, which mimics the pathologies in AD, not only by directly decreasing the dephosphorylation but also indirectly by promoting the activities of CaM kinase II [47], PKA [48], MAPK [49], and extracellular regulated kinase (ERK 1/2) [50]. Given these findings, increasing PP2A activity may be an effective pharmaceutical approach to inhibit the abnormal hyperphosphorylation of tau.

Tau Mechanisms in TBI and Neurodegeneration

It is becoming evident that brain injury can lead to chronic traumatic encephalopathy [15], which is considered a progressive neurodegenerative disease. In spite of many reports that implicate brain trauma as a risk factor for AD, these findings are predominantly based on clinical diagnostic criteria and a biomarker has yet to be identified [51]. Furthermore, the epidemiological association between TBI and AD is based on AD-like pathologies found in both patients and animal models of TBI [15, 52].

Several mechanisms may be involved in TBI associated-neurodegeneration, such as neuronal loss, persistent inflammation and cytoskeletal pathology. However, NFT have received increasing attention as one of the pathological links due to their characteristic presence in AD pathologies [27]. A recent report showed increased tau in the peri-contusion spaces in patients with severe TBI and the initial higher tau level was correlated with long-term cognitive impairments [53].

Abnormal increases in phosphorylated tau reduces its affinity for MTs resulting in the destabilization of cytoskeleton in the central nervous system [37, 54]. Hyperphosphorylated tau is pathologically detached from the MTs, aggregates into NFTs and accumulates in neurons, astrocytes or oligodendroglia, leading to axonal and synaptic dysfunction and neurodegeneration [29].

The importance of the reduced binding capacity of tau in neurodegeneration has recently been validated in in-vivo studies, which demonstrated that the MT stabilizing drugs could be used to slow or halt the progressive neurodegenerative phenotype of transgenic AD models [29]. However, the discovery that the severity of cognitive dysfunction was only correlated with the total level of NFTs, but not the levels of Aβ [55], provided circumstantial evidence to suggest that toxic NFTs might play a pivotal role in the progression of the neurodegenerative diseases [36]. Experimental evidence suggests that filamentous tau may not be exclusively responsible for neuronal dysfunction [36, 56].

Tau Mechanisms in Epilepsy

Thom et al [67] reported the finding of NFT pathology in patients who had temporal lobectomies for with chronic epilepsy, and associated this with long-term cognitive decline. About one third of patients were reported to have had a previous brain injury. Our group has recently reported that targeting hyperphosphorylated tau with the drug sodium selenate, which enhances the activity of PP2A, suppressed seizures in several rodent models of epilepsy, suggesting that this might become a novel approach to treatment of patients with epilepsy [66]. In addition, reduction of endogenous tau can

prevent premature mortality and increase the threshold to pentylenetetrazol (PTZ)-induced seizures in transgenic mice [57]. However, to date, there has been no report that specifically implicates tau-based mechanisms in PTE.

Disease-Modification Treatment Approaches Targeting Tau-Based Mechanisms

The function of tau is primarily regulated by phosphorylation and dephosphorylation, as discussed above. Therefore, most clinical and experimental studies are focused on the kinases or phosphatases of tau.

Kinase Based Treatment

Of the tau kinases targeted as potential treatment targets for brain injury and neurodegeneration, the most widely studied are GSK-3 beta [58], CDK [59], MARK [60], and MAPK [61]. Lithium, a GSK-3 beta enzyme inhibitor, has been reported to attenuate the neuronal degeneration and improve the cognitive outcome after experimental TBI [62, 63]. In a similar way, the CDK5 inhibitor, roscovitine, has been found to reduce the neuronal loss, glial activation and neurologic deficits after brain trauma [64, 65].

Phosphatase Based Treatment - PP2A

A single tau phosphatase, PP2A, accounts for over 70% of tau phosphatase activity in the human brain [46]. PP2A is widely expressed throughout the body and at high levels in the brain [66], where it plays a principal role in removing phosphate residues from tau [46]. PP2A promotes tau mediated stabilization of the MT and prevents the assembly of phosphorylated tau into paired helical filaments and NFTs [67]. PP2A exists as a functional heterotrimer, which consists of a catalytic C subunit, a scaffold A subunit, and a regulatory B subunit with variable isoforms. Up to now, four kinds of B subunits have been identified, termed the B, B', B'' and B''' families, and a different B subunit directs PP2A to different spectra of substrates and different subcellular compartments [68]. Only the Bα-containing isoform (ABαC) has been demonstrated to effectively bind to and dephosphorylate tau [69]. The underlying mechanism of selective interaction of the Bα subunit and phosphorylated tau has

been revealed by X-ray crystallography [70]. PP2A can indirectly regulate tau phosphorylation via activating GSK-3 beta [83].

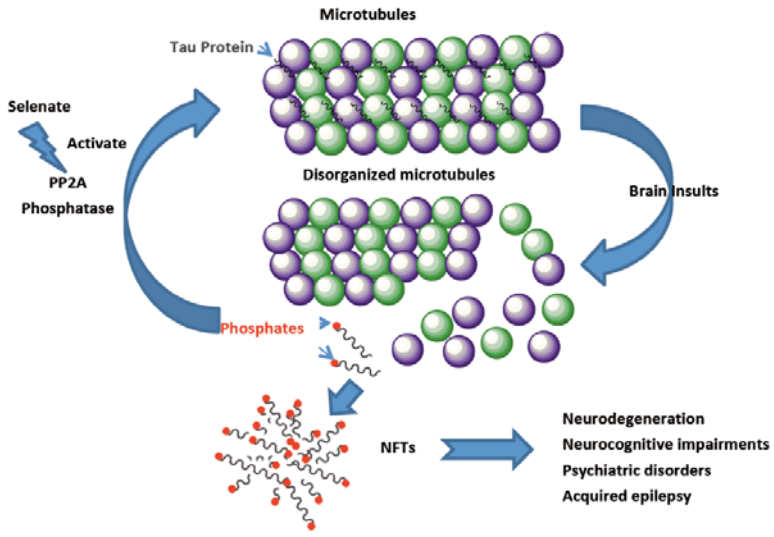

Fig 1. In the normal physiological condition, tau proteins are in a balance of binding or detaching to the microtubules (MTs). After brain insults, the MT become disorganized and most tau become phosphorylated and detached. In the pathological condition, these phosphorylated tau aggregate in to neurofibrillary tangles (NFTs) and result in the acquired epilepsy, neurodegeneration and neuropsychiatric comorbidities following brain insults. Sodium selenate increases the activity of PP2A (major phosphatase) and reverses the tau to bind to MTs and potentially mitigates these pathological processes.

PP2A Linkage to Neurodegeneration

Growing evidence implicates PP2A in a number of pathological mechanisms from carcinogenesis to neurodegenerative disease [71]. Decreased PP2A mRNA [72], protein [69] and phosphatase activity [45, 69] have been observed in postmortem brains from AD patients. Reduced immunoreactivity of neuronal ABαC (a main component of PP2A) is found to cause decreased activity of PP2A, which is closely correlated with NFT accumulation but not Aβ burden, suggesting that ABαC dysfunction contributes to tau pathology in AD models [68]. Enhancing the activity of PP2A is hypothesized to be an alternative therapeutic strategy for neurodegenerative diseases.

Based on many experimental studies, PP2A has been proven to be the major phosphatase for phosphorylated-tau [46]. Enhancing PP2A activity would be a potential target for therapies to suppress phosphorylated tau and NFT pathology [73].

PP2A Linkage to TBI

Chen et al [84] reported that phosphorylated tau levels increased very shortly following a TBI in a compression brain injury model after the onset from 10 min to 12 h, and this change resulted in a downregulation of PP2A activity and transient activation of tau kinases. However, whether this change will affect the cognitive outcome requires further study.

PP2A Linkage to Epilepsy

Pharmacological inhibition of PP2A with OA induces seizures [74] and many pathological features of AD were recapitulated including Aβ deposition, tau hyperphosphorylation [75] and cognitive deficits [75]. The effects of these kinds of PP2A inhibitors may not be selective, and other PPases including PP1, PP4-6 may be inhibited in these in vivo studies due to lack of control of the specific inhibitors in given brain regions. Experimentally, hyperphosphorylation of tau has been implicated in both seizure and epilepsy pathology [76], and lowering endogenous tau expression in AD mice reduces the seizure susceptibility [57].

Sodium Selenate – a PP2A Activator

##Using an in vitro malachite green assay to screen for compounds that boost the activity of a PP2A, sodium selenate (Na_2SeO_4) was found to markedly boost PP2A activity [77] (**Fig 1**). This was also observed in an alternative chemical substrate assay using para-nitrophenylphosphate (pNPP), increasing PP2A enzyme activity by 8% [77]. The related selenium compounds, inorganic sodium selenite (Na_2SeO_3) and organic selenomethionine, were unable to increase PP2A core activity [77].

Long-term treatment with sodium selenate for 3 months has been found to significantly decrease the levels of phosphorylated tau at AT180 and total tau (HT7) in the hippocampus and amygdala of P301L mice which expressed human mutant tau, compared to rodents treated with vehicle [77]. Curia et al [78] found injuries in these brain areas are more prone to the development of epileptogenesis than other brain structures.

Enhancing PP2A with Sodium Selenate Suppresses Seizures in Rodent Models

Recent work has reported that treatment with sodium selenate significantly reduced the frequency and duration of seizure activity in rodent models of epilepsy [79]. In unpublished work, our group has shown that selenate treatment progressively reduced seizure duration in the rat amygdala kindling model (Liu et al., manuscript in preparation). Western blotting demonstrated that kindled rats treated with sodium selenate had decreased phospho-tau and total-tau levels in brains compared to vehicle treated rats.

Other PP2A Activators

FTY720 (Fingolimod), which has recently been approved by the Food Drug Association (FDA) to treat multiple sclerosis [80], has been found to activate PP2A through increasing the expression of PP2A subunit B (PR55) [81], and can penetrate the blood-brain barrier and concentrate in the brain [82]. Nevertheless, whether it is able to decrease the phosphorylation of tau and suppress the spontaneous seizures in rodent models of epilepsy has not been studied.

Summary and future challenges

In this article, we have summarized recent research into the mechanisms and treatment of acquired epilepsy with a focus on tau-based mechanisms and opportunities for targeting these for novel disease-modifying therapy. The mechanistic link between brain insults and the later development of acquired epilepsy remains poorly understood, but one recurrently implicated mechanism is the involvement of tau protein and NFTs in brains. Proof-of-concept studies targeting hyperphosphorylated tau based mechanisms have proven to be effective in inhibiting seizures and neurodegeneration in a variety animal models. Whether the promising experimental studies in rodents are translatable to protective therapies against epilepsy and is associated neuropsychiatric and neurodegenerative co-morbidities in the clinical setting remains to be demonstrated.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Dear Editor,

I am sending you our manuscript entitled "**Hyperphosphorylated Tau is Implicated in Acquired Epilepsy and Neuropsychiatric Comorbidities** " by Zheng et al. We would like to have the manuscript considered for publication in "Molecular Neurobiology". As the original manuscript has been accepted for publication, however, other co-authors put more information and revision to the original. Therefore, we resubmitted our manuscript.

All authors have approved the manuscript and agree with its submission to

"Molecular Neurobiology".

Please let me know of your decision at your earliest convenience.

With our best regards,

Ping Zheng

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