

● PERSPECTIVE

Animal model of repetitive mild traumatic brain injury for human traumatic axonal injury and chronic traumatic encephalopathy

Chronic traumatic encephalopathy (CTE) is a chronic neurodegenerative disease featured with tauopathy. CTE is tightly related with repetitive mild traumatic brain injury (mTBI), which is interchangeably known as concussion (McKee et al., 2009, 2013). This disease is differentiated by neuropathological features from other neurological diseases that involve tau protein aggregation and tangle formation abnormalities like Alzheimer's disease (AD), frontotemporal dementia, and Parkinsonism linked to chromosome 17 (FTDP-17). The differentiation of CTE from other neurodegenerative diseases provides helps on exploring the mechanism of etiology and seeking potential treatment for this disease. Repetitive mTBI is usually evident in civilians participating in contact sports and in military personnel engaging in combat. Due to the number of individuals partaking in these high-risk environments, the general public concern has heightened in concordance to the reveal of many new CTE cases. Despite increased awareness, the mechanisms of CTE are still unknown and there are no effective therapies to help slow the progression of the devastating disease. The heterogeneity of human CTE cases and the complexity of patients' backgrounds make it difficult for scientists to specify the links between mTBI and CTE using epidemiological or clinical approaches; appropriate animal models are effective in exploring cause-and-effect relationships.

We developed this repetitive mTBI mouse model (IA model) (Xu et al., 2014) with the hope of clarifying relationships between multiple concussions and tauopathy, to clarify CTE mechanisms, and to eventually serve as vehicles for experimental therapeutics or for biomarker discovery and validation. The IA model incurs traumatic axonal injuries (TAI) approximate to the ones induced in contact-related repetitive concussions, which supports the notion that this model can be used as a tool to investigate repeated concussive events in human subjects. We did not find any signs of tauopathy in the mouse IA model after repetitive mTBI (Xu et al., 2014), even though tau hyperphosphorylation was evident in a single blast injury in another study experimenting on the same mouse strain (Goldstein et al., 2012).

The incidence and prevalence of CTE is currently still unknown. Even though there is a large population of repetitive mTBI victims (1.4–3.8 million mTBI annually in USA), only a small fraction of these cases are diagnosed as CTE (only a couple of hundred cases are identified from post-mortem analyses and reported) (Baugh et al., 2012; McKee et al., 2013). Therefore there must be a number of factors that have decisive roles in CTE formation; one suggested factor is the genetic risk of mutations leading to abnormal tau aggregation. A variety of transgenic mice, which carry mutant human tau genes that induce tauopathy, can be used with this IA model to investigate if repetitive mTBI triggers or expedites tau aggregation. These mutations may include, but not limited to P301S, V337M, and the deletion mutation Δ K280. Our studies with the P301S mouse resulted in significantly increased numbers of hyperphosphorylated tau in retina ganglion cells (RGCs) after repetitive mTBI.

However, there is no significant increased tau hyperphosphorylation in the cortex even though the corticospinal tract and corpus callosum were the major white matter TAI targets as optic tract in this IA model (Xu et al., 2014). One possible explanation for this finding is that cortical TAI is more diffusive than visual system TAI, and therefore does not generate experimental significance. In addition to P301S mouse, we observed similar results in tauopathy-prone transgenic mice, which harbored all six isoforms of the wild-type human tau gene but were knocked out for the mouse tau gene. Due to the absence of mouse tau, any possible interaction between mouse tau and human tau could be excluded in this transgenic mouse. These findings indicate that repetitive mTBI accelerates tauopathy under diverse number of genetic conditions that predispose a subject to tau aggregation; more transgenic mice with various mutations and with or without mouse tau need to be tested to confirm this theory. Other tau-related proteins should also be investigated to see if they contribute to abnormal tau aggregation. For example, evidence supports that aggregated A β protein, one feature of AD, increases tau phosphorylation and elevates the number of tangles in Tau p301L transgenic mice (Gotz et al., 2001). TBI is also one factor in inducing AD, but its causative role is still not confirmed. Therefore, transgenic mice that carry mutant amyloid precursor protein (APP), which then produces aggregate A β protein, can also help verify if repetitive mTBI expedites plaque formation in genetically vulnerable environments.

Other mechanisms may also be involved in tauopathy induction and progression after repetitive mTBI. Evidence supports that tau proteolysis plays an important role in tau aggregation; after TBI, excessive amounts of activated caspase and calpain were found (Knoblauch et al., 2002; Saatman et al., 2010), and these activated enzymes are capable of cleaving tau at various sites. The resulting truncated tau fragments form oligomers and trigger tau aggregation (Wang et al., 2007). Another tau protease, thrombin, mobilizes into the brain parenchyma through the TBI-disrupted blood brain barrier (BBB). Thrombin induces the proteolysis of tau proteins that leaked into the extracellular space after injury; these tau fragments following a pathological event may initiate tau aggregation as caspase or calpain mentioned above. In our repetitive mTBI model, significantly increased BBB permeability is evident in both single and repetitive injury paradigms. Therefore, questions regarding truncated tau and tau aggregation post-repetitive mTBI and also which proteases are most significant in tauopathy are still under investigation.

Neuroinflammation, which includes microglia activation, concomitantly occurs at the lesioned area after TBI. In our repetitive mTBI model, there is a significantly elevated number of activated microglia in the TAI location, although their specific role in repetitive concussive injury remains unclear. The activation of microglia from their resting, surveillance state can be detrimental and may contribute to a secondary injury. Activated microglia M1-type release a variety of pro-inflammatory and neurotoxic molecules such as reactive oxygen species (ROS) and tumor necrosis factor- α (TNF α), which induces further damage to the nervous system. Microglia also mediate retrograde axonal degeneration post-TBI (Busch et al., 2009), and chronic microglia activation has been linked to various neurodegenerative diseases like AD, amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD). Studies involving subjects with neurodegenerative diseases or TBI have shown that inhibiting microglial activation both clinically and experimentally with minocycline or other anti-neuroinflammatory therapies improve disease state (Sanchez Mejia et al., 2001; Homsí et al., 2010). In TBI, activated microglia can remain activated for a long period time,

sometimes from months to years (Gentleman et al., 2004; Johnson et al., 2013); this pattern indicates chronic neuroinflammation. CTE subjects have activated microglia in the tau tangle area, which suggests microglia may contribute to tauopathy severity. This relationship may exist since there is evidence to support that activated microglia is involved in tau phosphorylation and, perhaps, tau aggregation through interleukin1 (IL1)/p38 mitogen-activated protein kinases (MAPK) signaling (Bhaskar et al., 2010). Despite these hypotheses, the role of microglia in tauopathy induction and chronicity still needs to be confirmed; the mechanistic clarification of how microglia take part in tauopathy may lead to an effective therapy for CTE.

The development of effective therapies for TAI after concussion is still ongoing. The Wallerian degeneration slow (WLD^S) protein has experimental support for its protective effects on axons in experimental animal studies (Avery et al., 2012). Another therapeutic target may be dual leucine zipper kinase (DLK), which is a key mediator of axonal degeneration that is robustly upregulated after axonal injury; DLK inhibition can theoretically reduce retina ganglion cell (RGC) death caused by optic nerve transection (Welsbie et al., 2013). DLK is a kinase of c-Jun N-terminal kinase (JNK) and is the key activator of JNK signaling following axotomy. JNK is activated after TBI and activated JNK can lead to cell apoptosis (Greer et al., 2011). Vision disorder is also frequently experienced in TBI patients. In our repetitive mTBI model, the optic tract is one of the main white matter targets that show significant axonal injury; this localization thus led to distinct RGC death (Xu et al., 2014). Our pilot study showed that multiple mTBI can dramatically increase JNK in the RGCs. Therefore, blocking or disrupting DLK/JNK signaling may protect axons from degeneration and also improve the chances for RGCs survival. Floxed DLK or JNK transgenic mouse and corresponding pharmacologic reagents are now being used with our repetitive mTBI model to verify this therapeutic potential. If the pharmaceutical affectability is validated in the visual system, the next study will focus on cortical TAI, which is another major target for concussion therapy.

In summary, this repetitive mTBI model can be served as a tool for a wide range of both basic and translational repetitive concussion studies, due to its ability to replicate the traumatic nature of human concussion.

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