REVIEW

Remodeling dendritic spines for treatment of traumatic brain injury

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Abstract
Traumatic brain injury is an important global public health problem. Traumatic brain injury not only causes neural cell death, but also induces dendritic spine degeneration. Spared neurons from cell death in the injured brain may exhibit dendrite damage, dendritic spine degeneration, mature spine loss, synapse loss, and impairment of activity. Dendritic degeneration and synapse loss may significantly contribute to functional impairments and neurological disorders following traumatic brain injury. Normal function of the nervous system depends on maintenance of the functionally intact synaptic connections between the presynaptic and postsynaptic spines from neurons and their target cells. During synaptic plasticity, the numbers and shapes of dendritic spines undergo dynamic reorganization. Enlargement of spine heads and the formation and stabilization of new spines are associated with long-term potentiation, while spine shrinkage and retraction are associated with long-term depression. Consolidation of memory is associated with remodeling and growth of preexisting synapses and the formation of new synapses. To date, there is no effective treatment to prevent dendritic degeneration and synapse loss. This review outlines the current data related to treatments targeting dendritic spines that propose to enhance spine remodeling and improve functional recovery after traumatic brain injury. The mechanisms underlying proposed beneficial effects of therapy targeting dendritic spines remain elusive, possibly including blocking activation of Cofilin induced by beta amyloid, Ras activation, and inhibition of GSK-3 signaling pathway. Further understanding of the molecular and cellular mechanisms underlying synaptic degeneration/loss following traumatic brain injury will advance the understanding of the pathophysiology induced by traumatic brain injury and may lead to the development of novel treatments for traumatic brain injury.

Key Words: *traumatic brain injury; dendritic spines; synaptic plasticity; spinogenic agents; treatment; spine remodeling; memory; functional recovery*

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Traumatic Brain Injury
Traumatic brain injury (TBI) is a significant public health problem worldwide. Everyone is at risk, particularly the very young and the elderly, athletes of all ages who participate in contact sports, military personnel in the field of combat, automobile drivers, passengers and pedestrians. TBI comes in various degrees of severity, from mild to moderate to severe. TBI results in structural damage and functional deficits due to both primary and secondary injury mechanisms. Primary injury that occurs at the time of trauma is the direct result of the external mechanical forces that produce deformation of the brain tissue (contusion, damage to blood vessels, and axonal shearing) and disrupt normal brain function (Xiong et al., 2013). Secondary injury that evolves over minutes to months following the primary injury results from a multifactorial set of biochemical events including glutamate excitotoxicity, perturbation of cellular calcium homeostasis, membrane depolarization, mitochondrial dysfunction, inflammation, increased free radical generation and lipid peroxidation, apoptosis, autophagy, and diffuse axonal injury (DAI). These complex cascades lead to ischemic and hypoxic damage, cerebral edema, raised intracranial pressure, cell death, brain atrophy, and functional deficits (Xiong et al., 2013). Animal models of TBI are essential for studying the biomechanical, cellular, molecular and behavioral aspects of human TBI as well as for developing novel therapeutic interventions that cannot be directly investigated in the clinical TBI (Xiong et al., 2013). Larger animals have gyrencephalic brains that are closer in size and physiology to humans and have been increasingly used for the preclinical study of TBI. Lissencephalic rodents are most frequently used in TBI research because of their modest cost, small size, easy genetic manipulation, and availability of standardized functional outcome measurements among other reasons. Increasing evidence from animal studies demonstrates that TBI-induced functional deficits are closely related to damage to the cellular projections of neurons termed dendrites and axons. Microtubules and neurofilaments, containing cytoskeleton proteins, in dendrites and axons are required for neuronal function. The physical forces that occur when the brain is rapidly accelerated, decelerated or rotated can disrupt these cytoskeleton proteins. DAI is a hallmark of TBI pathology and happens when the axons are sheared as the brain rapidly accelerates and decelerates inside the hard bone of the skull. In addition to cell death and DAI, extensive dendritic damage in the spared neurons may disrupt neurocircuits and significantly contribute to functional impairment following TBI (Mulherkar et al., 2017). Dendritic spines are small protruding structures on dendritic surfaces, and function as postsynaptic compartments for excitatory

synapses. Plasticity of spine structure is associated learning and memory. Microtubules together with actin play an important role in the control and regulation of dendritic spine morphology and synaptic plasticity. Microtubules and actin are vulnerable to misalignment and dissolution caused by injury, which may cause dysregulation of spine morphology, dynamics, and synaptic plasticity resulting in subsequent functional impairment after TBI (Mulherkar et al., 2017). We have performed a PubMed literature search of articles published in the period of March 1995-November 2018 with the keywords of "traumatic brain injury; dendritic spines".

Dendritic Spine Degeneration and Loss after Traumatic Brain Injury

Learning and memory deficits are frequent hallmarks of brain injury associated with hippocampal damage and are the most enduring and devastating consequences following TBI. TBI induced by a moderate level of controlled cortical impact (CCI) in mice causes both immature newborn neuron death in the hippocampal dentate gyrus and mature neuron loss in the CA3 and dentate gyrus (Gao et al., 2011). Although most of the mature granular neurons are spared following TBI at a moderate level of impact, they exhibit dendritic beading and fragmentation, decreased number of dendritic branches, and reduced dendritic spine density, particularly the mushroom-shaped mature spines, suggesting spared mature neurons are compromised by TBI (Gao et al., 2011). The reduced density of synapses in the molecular layer of the hippocampal dentate gyrus is associated with the impaired electrophysiological activity of the neurons. These results indicate that moderate TBI not only induces cell death in immature granular neurons but also causes significant dendritic and synaptic degeneration in spared mature neurons in the hippocampal dentate gyrus. Unlike moderate and severe TBI, mild TBI (mTBI) does not evoke significant tissue lesions or cavities in the cortex. TBI, especially repetitive mTBI, can lead to long-term cognitive and emotional difficulties and behavioral disturbances. mTBI induced by a lateral fluid percussion injury in rats does not reduce neuronal numbers in the infralimbic cortex, but causes a significant reduction in overall dendritic spine density of both basal and apical dendrites in layer II/III pyramidal neurons (Zhao et al., 2018). The reduction in spine density in layer II/III pyramidal neurons is associated with impairment of contextual fear memory extinction (Zhao et al., 2018). These studies suggest that dendritic degeneration that occurs at the subcellular level may be an important target for developing therapeutic approaches for TBI.

Targeting Dendritic Spines for Treatment of Traumatic Brain Injury

Neuroprotective therapeutics targeting cell death in TBI have failed in clinical trials, including anti-inflammatory drugs, hyperbaric oxygen, progesterone and many other treatments. Preventing spine loss/promoting spine remodeling may open a new avenue for treatment of TBI (**Figures 1** and **2**). For example, excessive glutamate signaling after TBI can induce calcium overload to activate calcium-sensitive phosphatase calcineurin (CaN), and an increase in CaN activity alters its downstream effector cofilin an actin-depolymerizing protein to lead to dendritic spine loss. Dephosphorylation of p-Cofilin promotes actin depolymerization. Amyloid-beta (Aβ) aggregates induce spine loss through a pathway that involves activation of Cofilin-dependent depolymerization of actin upon binding of Aβ aggregates to the high-affinity PirB receptor for Aβ. A single post-traumatic administration of the CaN inhibitor FK506 reduces loss of spines in rats after TBI (Campbell et al., 2012). A small molecule 7,8-dihydroxyflavone that mimics the function of brain-derived neurotrophic factor through activating the TrkB-mediated PI3K/Akt signal pathway, reduces dendrite swelling in the cortex and also prevents dendritic spine loss after TBI as well as improves rotarod performance (Zhao et al., 2016). Microtubule dynamics underpin a plethora of roles involved in the intricacies of neuronal development, structure, function, and maintenance. Within the injured brain, microtubules are vulnerable to misalignment and dissolution in neurons. A single low dose of the brain-penetrant microtubule-stabilizing agent epothilone D administered immediately following TBI significantly decreases spine length and increases density of mushroom spines in the dendritic spines of layer V cortical projection neurons (Chuckowree et al., 2018). In neurons, the Rho GTPase Rac1 promotes the growth of axons and dendrites and the formation and maintenance of spines/synapses, whereas RhoA induces axonal and dendritic retraction and spine/synapse loss. Excessive RhoA activation may cause spine and synapse loss observed after TBI. Fasudil inhibits RhoA-ROCK signaling and alleviates deficits in motor and cognitive performance and prevents TBI-induced mature spine loss in a mouse CCI TBI model (Mulherkar et al., 2017). Acute inhibition of RhoA-ROCK signaling attenuates the detrimental effects of TBI on motor coordination and balance and prevents hippocampal-dependent contextual fear discrimination impairment (Mulherkar et al., 2017). Treatment with N-acetyl-seryl-aspartyl-lysyl-proline an active peptide fragment of thymosin beta 4 significantly improves sensorimotor functional recovery and spatial learning and increases the number of dendritic spines in the injured brain (Zhang et al., 2017). These data suggest that treatments that enhance dendritic plasticity may contribute to improved functional recovery after TBI.

Novel Spinogenic Compounds for Treatment of Traumatic Brain Injury

The majority of excitatory synapses in the brain are present on dendritic spines. Dendritic spines are critical to the formation of synapses that play important roles in neuronal circuits, learning, and memory. Developing novel spinogenic molecules is warranted to address key aspects of the im-

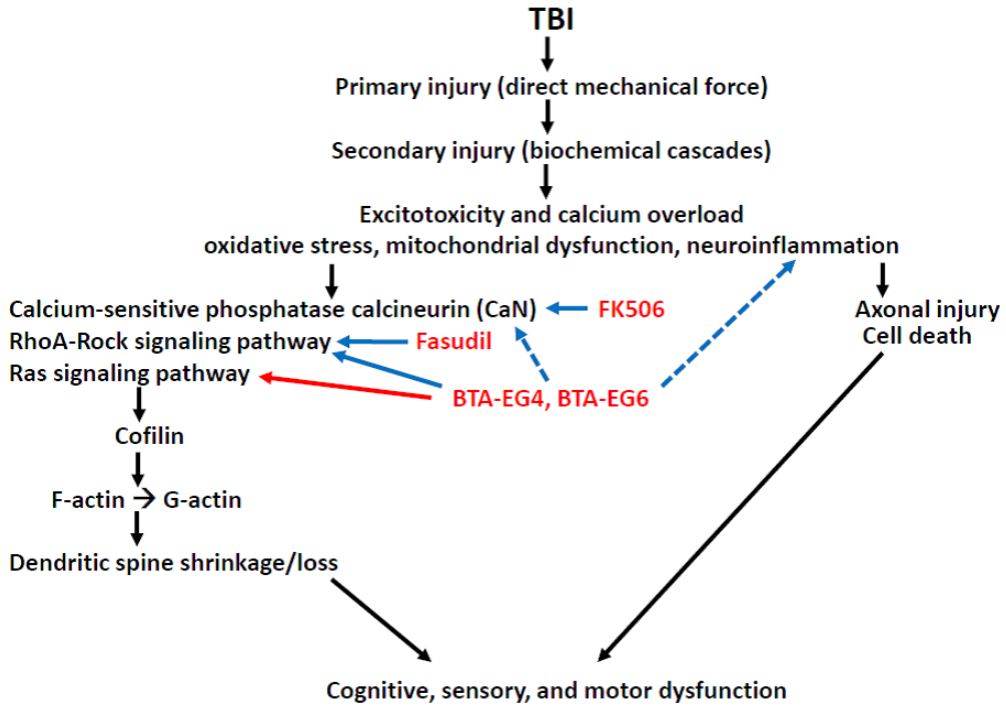

Figure 1 Schematic representation of spine loss and functional dysfunction following traumatic brain injury (TBI).
The primary injury force is a direct physical force or rotational acceleration/deceleration that causes either a focal or diffuse brain injury which then initiates secondary biochemical injury mechanisms leading to axonal injury, cell death, and impaired synaptic plasticity that contribute to the functional dysfunction after TBI. Solid blue lines: Inhibition; dotted blue lines: effects that need investigation; solid red lines: activation.

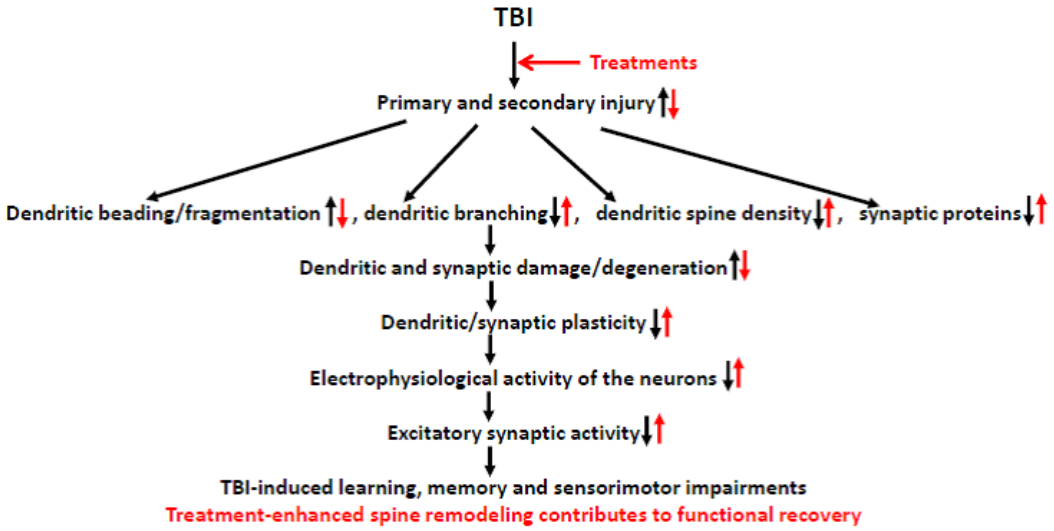

Figure 2 Schematic of treatment-induced dendritic spine remodeling after traumatic brain injury (TBI).
Solid black arrows: TBI detrimental effects; solid red arrows: treatment beneficial effects.

mediate and long-term effects of TBI including generation of toxic Aβ, loss of spine density in critical brain areas and impaired memory and concentration. Derivatives (benzothiazoleaniline, BTA) of the thioflavin-T series bind to Aβ with high affinity. One of the early spinogenic molecules BTA-EG4 (aka SPG101) improves memory and reduces Aβ in triple transgenic Alzheimer's disease mice and exhibits a dose-dependent response leading to an increase in dendritic spine density in primary hippocampal neurons (Song et al., 2014). A new BTA-EG4 analog BTA-EG6 blocks the Aβ-induced activation of Cofilin, thereby reducing Aβ-induced spine loss (Cifelli et al., 2016). BTA-EG4 promotes a net increase in spine density through the formation of new spines through amyloid precursor protein-and Ras-dependent mechanisms (Megill et al., 2013). BTA-EG4 can penetrate the blood-brain barrier and protect neurons from Aβ-induced toxicity (Song et al., 2014). Whether it restores spine density in the critical brain areas (cortical layers 2/3, hippocampus, and dentate gyrus) impacted by TBI remains unknown. Our recent study investigated the therapeutic effects of BTA-EG4 on dendritic spine density and morphology and functional recovery in a rat model of TBI induced by CCI (Zhang et al., 2018). Young adult male Wistar rats subjected to CCI were intraperitoneally administered with BTA-EG4 (30 mg/kg) dissolved in vehicle (dimethyl sulfoxide in phosphate buffered saline) or Vehicle starting at 1 hour post-injury and once daily for the next 34 days. Compared with the treatment control, BTA-EG4 treatment

significantly improved sensorimotor functional recovery, spatial learning in the Morris water test, novel object recognition, and social recognition. The brains were processed for 35 days after injury for measurement of dendritic spine density and morphology using ballistic dye labeling. SPG101 treatment significantly increased dendritic spine density in the injured cortex and decreased heterogeneous distribution of spine lengths in the injured cortex and hippocampus. These spine modifications are associated with the promotion of spine maturation in these brain regions. Our data suggest that treatment with BTA-EG4 initiated 1 hour post-injury and continued for an additional 34 days improves both sensorimotor and cognitive functional recovery suggesting that BTA-EG4 acts as a spinogenic agent and may have potential as a novel treatment for TBI and possibly for many other diseases with spine loss.

Future Directions of Research on Spinogenic Compounds for Treatment of Traumatic Brain Injury

Our BTA-EG4 study (Zhang et al., 2019) is a relatively acute and long-term treatment study. Future studies are warranted to investigate: 1) effects of different doses on functional recovery and spine remodeling; 2) effects of delayed treatments with BTA-EG4 because early treatment within 1 hour may not be practical in clinical settings; 3) effects of BTA-EG4 on many other important aspects of neurovascular remodeling including angiogenesis, neurogenesis and remyelination that are involved in TBI recovery; 4) roles of age and sex on BTA-EG4 effects; and 5) effect of BTA-EG4 in different animal TBI models, because TBI in the clinical setting is a heterogeneous injury.

Dendritic spines are small protrusions in neuronal dendrites where the postsynaptic components of most excitatory synapses reside. Precise regulation of dendritic spine morphology and density is critical for normal brain function. Abnormal spine morphology is linked to many neurological diseases and injury including TBI. The actin cytoskeleton is a structural element underlying the proper morphology of dendritic spines. It is generally believed that the actin cytoskeleton resides only in dendritic spines and controls spine morphology and plasticity. Microtubules, also present in spines, especially in mushroom-shaped mature spines, may play an important role in spine development and dynamics. Further studies are warranted to investigate effects of BTA-EG4 on neuronal cytoskeleton components including actin and microtubules, and to elucidate mechanisms underlying the beneficial effects of BTA-EG4 including but not limited to Aβ, Tau, and signaling pathways such as the Ras and RhoA-ROCK signaling pathways after TBI. Development of next generation spinogenic compounds with better solubility and efficacy, and less side effects is also warranted. Spinogenic agents provide new tools to study the relationship between dendritic spines and cognitive behavior and may lead to novel approaches for the treatment of TBI and other dendritic spine-related cognitive disorders.

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