

Review Article

Epigenetics of Neurotrauma

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Abstract

Epigenetic changes have been linked to a host of disease states. Besides the physiological function of epigenetic changes in regulating cellular function, recent data indicates that key changes in epigenetic activity also play an important pathophysiologic role following neurotrauma specifically. Such manifestations occur through the activation or silencing of different genes. Histone methylation has emerged as a critical component of this process and can be selectively modulated after injury. Pre-clinical studies have resulted in key discoveries regarding specific methylation sites of interest. This focused review highlights some of these early findings and their relationship to clinical outcomes. These findings suggest areas of future investigation and discovery in the quest to develop ideal biomarkers and methods to utilize them in developing therapeutic interventions.

Keywords: Neurotrauma; Epigenetics; Biomarker; Methylation; Targets

Background

Epigenetic alterations are implicated in neurodevelopmental processes and linked to a variety of brain pathologies [1-8]. Epigenetics can help us better understand the molecular pathways that cause brain damage, react acutely to initiate healing responses, and discover targets for effective therapeutic intervention to help neurons adapt and survive. Intracellular epigenetic changes in response to environmental stimuli occur through well-known processes such as DNA methylation, hydroxy-methylation, histone modification, and non-coding RNA gene expression [3,6,7,9-13].

DNA methylation silences genes by interfering with transcription factor binding to DNA [3,7,8,12,14-16]. In this process, DNA Methyltransferases (DNMT) donates methyl groups at dense CG dinucleotide sequences in DNA called CpG islands [14]. DNA methylation patterns in the brain may be a possible marker to predict progression of neurodegenerative disorders that impact normal aging and cognitive processes [8,12,15,17]. Since DNMTs are highly expressed in the nervous system, and their regulation of transcription facilitates synaptic plasticity, they are also involved in numerous cognitive processes and changes in brain function with aging. On the

other hand, hydroxy-methylation, which converts 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), is linked to increased transcriptional activity and prevents DNMT from interacting with euchromatin, playing a role in DNA repair, neuronal aging, and synaptic plasticity in the Central Nervous System (CNS) [3,18].

Histones are complex proteins with an overall basic pH that binds tightly to (acidic) DNA. This action limits access to the transcriptional machinery, and therefore gene expression. Methylation, acetylation, ubiquitination, citrullination, and ADP-ribosylation of histones modify DNA-histone affinity, releasing the DNA sequences and offering space for transcription to occur [3,4,7,8,15,16]. Histone Methyltransferases (HMTs) catalyze methylation, which results in gene suppression. Meanwhile, Histone Acetylation *via* Acetyltransferases (HATs) promotes gene expression owing to the electrostatic decrease between histones and DNA, resulting in euchromatin conformation [3,7,15,16,19]. Alterations to the balance of these enzymatic processes have major repercussions for overall brain health. For example, dysregulation of histone methylation is associated with cognitive decline and neurological illnesses; dysregulated histone acetylation has been linked to abnormalities in axon growth, oxidative stress, synaptic plasticity, and cognition [16,20,21].

Non-coding RNAs (ncRNAs), such as microRNA (miRNA), long non-coding RNA, and short-interfering RNA, are functional molecules that regulate gene expression at the post-transcriptional, translational, and post-translational levels by modifying RNA stability [7,13,15,22]. These mechanisms play an important role in normal brain development and function. The expansive repertoire of ncRNA sequences modifies mRNA coding for numerous proteins that in turn regulate entire signaling pathways. As such, maladaptive expression of ncRNAs in response to a traumatic stimulus or a change

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in the microenvironment can contribute to neurological disorders by compromising neuronal signaling, synaptic plasticity, and neural repair mechanisms [13,15,22].

Traumatic Brain Injury (TBI) is an insult to the brain caused by a mechanical impact [1,5,9,23]. TBI can affect memory, behavior, and neuropsychological status [7,9,13,24]. Consequences of the molecular mechanisms driving these symptoms are characterized as either primary and secondary injury; the primary injury occurs immediately after the initial traumatic insult and includes axonal shearing and intracranial bleeding, while secondary injury develops hours to days after the initial traumatic insult and includes biochemical disruption and cellular stress during the recovery phase [1,9,23-25]. The primary insult in the brain parenchyma generates a distortion in the lipid bilayer, resulting in potassium efflux and inflow of sodium, calcium, and chloride across the disturbed cellular membrane, which compromises Blood-Brain-Barrier (BBB) integrity, depending on the severity of the trauma, in many cases [9,25]. Following the original mechanical event, cellular dysfunction is caused by four primary mechanisms: 1) inflammation, 2) increased production of reactive oxygen and nitrogen-free radicals as well as oxidative damage, 3) ion-mediated damage (e.g., calcium), and 4) receptor-mediated dysfunction [9,13]. The rupture of the BBB and the overproduction of reactive species that cause oxidative stress, neuronal cell death, and altered proteome profiles that impact general protein activities begin several hours to days after the initial injury [1,9]. These processes, in turn, affect gene expression, which results in maladaptive responses and exacerbation of brain damage. Together, these events contribute to the long-term sequelae of TBI whereby patients have an elevated risk of developing neurodegenerative disorders in later life.

Neurotrauma is the largest environmental risk factor for developing Neurodegenerative Disorders (NDD) such as Alzheimer's disease and related disorders, movement disorders, and chronic traumatic encephalopathy, among others [7,9,26-30]. In underdeveloped countries, approximately 1% of the population has suffered a brain injury [26]. Because of the possible severity of the trauma and its potential short- and long-term effects on cognitive function, it is critical to use targeted treatments and preventative therapies by better understanding the cellular changes that occur in response to such events [31]. Because patients with TBI are more likely to develop NDD, advanced genomic and proteomic platforms are used to investigate the pathophysiological mechanisms. These studies uncovered numerous epigenetic changes leading to altered gene expression and post-transcriptional and post-translational protein modifications following TBI; these phenomena are similar to those seen in neurodegenerative processes [9,10,12,16]. Neuro-proteomic and epigenetic approaches applied to CNS injury can aid in evaluating novel pathways and mechanisms relevant to TBI pathogenesis. In doing so, it is important to identify important proteins as prospective biomarkers and potential drug targets [1,3,6,7,32]. This review aims to highlight and summarize current findings about cellular epigenetic changes following neurotrauma during both primary and secondary injury, with the goal of determining shared similarities with known neurodegenerative processes. Herein, we aim to draw attention to current interventions and clinical knowledge that can be supplemented by future clinical studies assessing targeted therapies for slowing neurodegenerative progression in neurotrauma patients both in the short and long term.

Pre-clinical investigations

Several recent pre-clinical models, particularly regarding hippocampal perturbations, have observed epigenetic modifications following induction of neurological trauma, including histone methylation, histone acetylation, and DNA methylation (Figure 1) [7]. Schober and colleagues examined the association of TBI and Insulin-Like Growth factor (IGF-1B) levels (a variant of the neuroprotective IGF-1) and found that epigenetic modifications were involved in the alterations of its serum levels post-injury [33]. Using a Controlled Cortical Impact (CCI) model of TBI in Sprague-Dawley rats, IGF-1B mRNA levels were increased significantly and correlated positively with specific histone modifications in the rat hippocampus associated with exon splicing [33]. In particular, increased IGF-1B mRNA was associated with methylation of promoter site 1, and other histone modifications associated with gene expression at promoter site 2, as well as differential methylation of the exon 5 exon-splicing enhancer [33]. Similarly, Wang and colleagues examined a murine CCI model of TBI and post-trauma treatment with Selective Serotonin Reuptake Inhibitors (SSRIs). They found increased histone 3 acetylation, as well as induction of methyl-CpG-binding protein, a DNA methylation transcription factor [34]. Gao and Colleagues similarly identified decreased histone 3 acetylation, along with decreased histone 3 methylation of hippocampal neurons [35].

Further examination of epigenetic alterations associated with TBI by Haghighi and colleagues assessed the frontal cortex in Long-Evans hooded rats in a blast-related TBI model [36]. In this model, rats were exposed to three 74.5 kPa blast waves one insult per day. Methylation profiles for both neurons and glia of the frontal cortex were analyzed. Their analysis identified 458 neuronal and 379 glial genes differing in their methylation patterns, relative to sham blast controls. Of particular interest, the neuronal genes altered were involved in cell death, cell survival, and CNS development and function. A 1.2-fold change of expression for the *serotonin N-acetyltransferase gene* was observed following increased methylation, and its biochemical involvement in both circadian rhythm and depression may align with changes observed in clinical TBI. Such findings provide an intriguing example of functional change that may result from post-TBI epigenetic alterations. Overall, these experiments demonstrate that epigenetic alterations observed in TBI may provide a connection between the clinical characteristics and their biochemical and metabolic underpinnings.

Clinical Correlates

The effects of TBI on gene expression and protein clearance are broad; however, much of the research on this topic surrounds Amyloid Precursor Protein (APP) and tau, specifically. Encoded by the Microtubule Associated Protein Tau gene (MAPT), tau is a heat stable protein that primarily plays a role in microtubule stabilization, allowing for bidirectional transport in axons [37,38]. Recent studies that identified tau interactomes in different models and organelles lead to the emerging concept that tau plays many as yet undefined roles besides those related to its association with microtubules [39-43]. For example, tau plays a role in mitochondrial activity and oxidative phosphorylation [43-45]. Neurofibrillary Tangles (NFT) form when aberrantly phosphorylated tau proteins aggregate [46]. These aggregates are present in several neurodegenerative disorders, including those that result from repetitive TBI such as chronic traumatic encephalopathy [46,47].

The *APP gene* (APPg) encodes for Amyloid Precursor Protein (APP), a Trans membrane protein that interacts with several elements

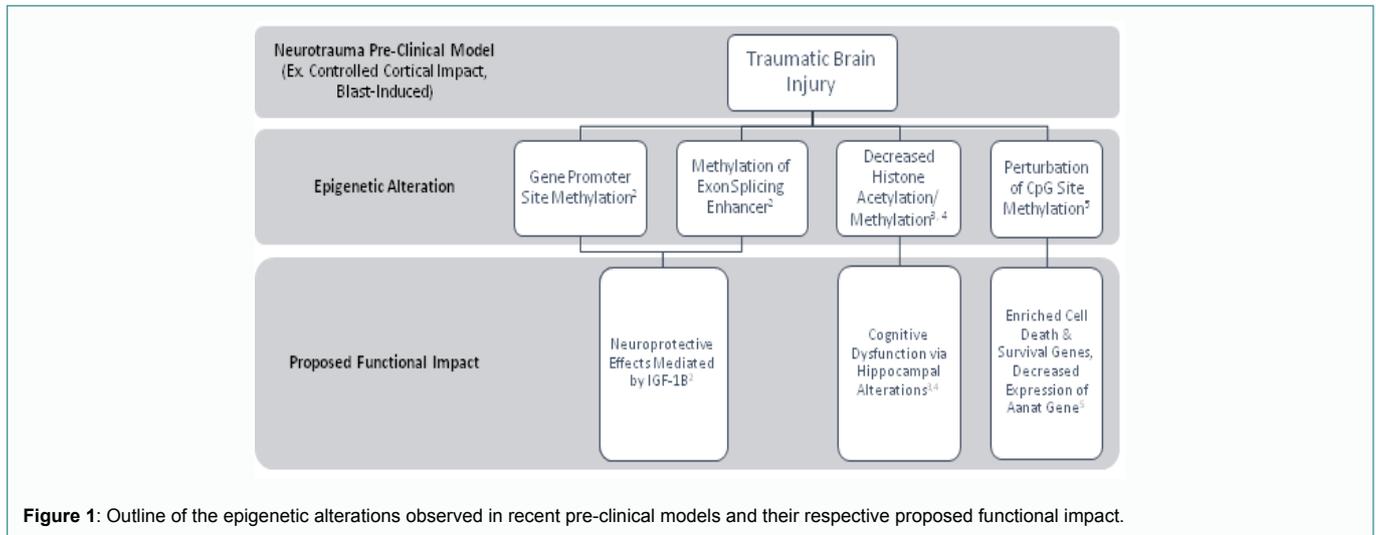


Figure 1: Outline of the epigenetic alterations observed in recent pre-clinical models and their respective proposed functional impact.

of the extracellular matrix [48]. It is thought that APP plays a role in cell-matrix and cell-cell adhesion [48]. Processing of APP also leads to the formation of Amyloid beta ($A\beta$), a 40 to 42 peptide protein fragment whose deposition is strongly tied to the development of Alzheimer's disease (AD) [49]. In the field of TBI, APP is primarily used as a surrogate of protein release from cells into plasma (it is acutely elevated after TBI) as a consequence of the trauma. However, due to the variability of its levels, APP is an unreliable biomarker of TBI.

While TBI is associated with increased risk for Alzheimer's disease, the significance of risk for developing dementia engendered by survivors of TBI is not clear, but a history of TBI has been significantly associated with risk factors for dementia [50]. More conspicuous associations include synucleinopathies. However, the mechanism driving the association of TBI with synuclein abnormalities remains unclear.

As APP and tau are hallmarks of AD pathology, and TBI is associated with increased risk of Alzheimer's disease. However, the underlying mechanisms that explain this association are not clearly elucidated. Studies have shown that expression of APP and Tau can be significantly impacted in the period following TBI [51]. Animal models have shown escalation of amyloid-beta ($A\beta$) and tau deposition following TBI but it remains unclear if the increased expression is pathologic and initiates progressive deposition [52]. Significance of deposition also seems to increase over time, with pathology presenting in distant areas that are synaptically linked to sites of initial damage [54].

Following neurotrauma, glial and neurotrophic factors are upregulated, as are other mediators of development to allow for recovery [54]. One of the ways this is accomplished is through epigenetic modifications, such as changes in histone-modifying enzymes that facilitate changes in gene expression in surviving cells, allowing for the CNS to adjust [54]. A recent study investigating epigenetic changes in patients with neurotrauma discovered differences in methylation of the APP, MAPT, Neurofilament Heavy (NFH), and Neurofilament Light (NFL) genes [10]. The study also showed more significant methylation of the APP gene which results in rapid accumulation and consequent $A\beta$ formation after TBI [10].

Methylation, catalyzed by DNMTs, represses gene expression

by inhibiting the attachment of transcriptional activators [55]. Abnormal patterns of DNA methylation are associated with cognitive difficulty as is seen in diseases of dysfunctional methylation such as Rett syndrome, Fragile X syndrome, and hereditary sensory and autonomic neuropathy type 1 (HSAN1), in which patients develop dementia and hearing loss in adulthood [55].

In the context of AD, studies have shown anomalous CpG methylation of the APP, MAPT, and GSK3B genes in patient brains post-mortem [56]. This connection between DNA methylation as a means of CNS recovery in the response following neurotrauma may elucidate pathway to better our understanding of the links between neurotrauma and neurodegenerative disease and the injury and repair responses.

Outcomes

TBI is a leading cause of death and long-term disability with increasing incidence and has led to increased numbers of emergency department visits and hospitalizations in recent years [3,23]. Epigenetic mechanisms occurring in response to TBI are the focus of an increasing number of studies [6,7]. The regenerative efforts mediated by epigenetic modifications could be transient or long lasting, and they may contribute to both short- and long-term outcomes following injury.

Health outcomes following neurotrauma are complex and often unpredictable. This is likely because TBI involves a spectrum of insults that range from mild-moderate-severe and with heterogeneity in location, severity, and symptom development [3,57]. A number of mechanisms contribute to the varied pathophysiologic responses to injury, and symptoms can appear immediately after injury or days to weeks after with substantial variability [24,26,31]. This is a major challenge to determining an appropriate course of effective care. Epigenetic changes may underlie many of the complex biochemical changes involved in injury following neurotrauma in the acute phase and may affect recovery in the longer term. Discovering epigenetic modifications maladaptive to recovery following injury could help in the development of therapeutics aimed at addressing physical and psychological effects of injury [1].

TBI induces metabolic dysfunction in the CNS and may cause short-term consequences that resolve quickly. Longer lasting symptoms and neuropsychiatric consequences include headache,

sleep disturbances, depression and anxiety, deficits in learning and memory, and behavioral defects. Further, the progressive nature of certain injuries puts patients at increased risk for long lasting neurodegenerative consequences such as Parkinson's Disease and Alzheimer's disease [7,13,58]. Epigenetic responses regulate many important cell functions and contribute to neural plasticity; thus, it is critical to understand how modified cellular metabolism following neurotrauma impacts epigenetics and *vice-versa*. These mechanisms could be targeted therapeutically to mitigate negative outcomes and enhance reparative mechanisms.

Interventions

Although the traumatic damage caused by TBI occurs in less than 100 msec, the effects of TBI can be lifelong. The persistence of the clinical symptoms enumerated above suggests that epigenetic modifications to cellular function may play a role. While epigenetic modifications can be long lasting, they can also be reversed [7]. Thus, examining them in the context of TBI creates opportunities for developing novel therapies for the treatment of TBI [7].

Support for clinical implementation of epigenetic modifications comes from various preclinical models. Using a CCI model, Gao et al. [35] reported hippocampal CA3 histone H3 methylation decreases at a 6-hour post-injury time point and lasted at least 72 hours post-injury [35]. This study suggested that epigenetic changes to the hippocampus play a role in cognitive impairment after TBI, and as such, targeting histone methylation becomes a potential therapeutic pathway. Another study highlighted that TBI increases both histone methylation and acetylation and DNA methylation in the *IGF-1* gene [33]. These changes led to an increased expression of an alternative form of *IGF-1*. *IGF-1* plays an important role in neuronal repair and regenerative efforts. Thus epigenetic modifications can produce both positive and negative effects after TBI-an important consideration when looking for therapeutic applications.

Similar to nucleic DNA, mitochondrial DNA (mtDNA) can also be epigenetically modified and impact bioenergetics of the cells. Under stress conditions such as those induced by TBI, diminished mitochondrial capacity to encode crucial electron transport protein can contribute to worsened outcomes [6,30,44,59,60]. MicroRNAs regulate aspects of mitochondrial function including mitochondrial gene expression associated with apoptosis and oxidative stress responses [59]. Consequently, modulation of cell bioenergetics and targeting the level of mitochondrial DNA methylation are growing areas of interest, with the potential to lead to novel therapies *via* modulation of the miRNA and mitochondrial DNA methyltransferase (mDNMT) enzymes [61,62]. In an *in vitro* study of focal ischemia, miR-29c levels decreased two-fold after the introduction of Oxygen-Glucose Deprivation (OGD) [63]. Pandi et al. [63] showed that pretreatment with pre-miR-29c increased post-OGD levels of miR-29c and halved cell-death [63]. Lundberg and colleagues described the impact of TBI on the expression of DNMT [64] and found that at four days post-CCI injury, DNMT-1 was co-expressed with Glial Fibrillary Acidic Protein (GFAP) and nestin, an indicator that cells have transitioned to a less differentiated state. Subsequently, Lundberg et al. [64] argue that by selectively derepressing transcription, DNMT-1 may induce expression of specific genes that are critical to astrocyte differentiation. As a result, DNMT-1 may contribute to the epigenetic reprogramming of *in situ* reactive astrocytes, a reaction precipitated by TBI, providing an explanation for the persistence of astrogliosis. This process could potentially be targeted to improve outcomes.

In their comprehensive review of epigenetic modifications, Chen et al. [65] note that the epigenetic modification of nucleic acids is a rapidly growing area of study [65]. With new technologies, such as single-base or single-cell resolution mapping strategies, and newly identified epigenetic markers, researchers see the opportunity to widen the scope and impact of nucleic acid modifications. Nucleic acids have significant roles in all living systems but elucidating their post-injury modifications indicates that they have a bigger role than previously thought. Specifically, nucleic acids may also have regulatory functions in numerous biological processes. For example; DNA methylation occurs throughout the entire genome and typically affects CpG islands in promoters [66]. Subsequently, methylation-binding proteins bind to DNA resulting in gene repression. As such, DNA methylation and demethylation are both associated with gene expression regulation [65]. This highlights the role epigenetic modifications have on transcriptional programs and, by extension, cell fate. Together, these studies create foundational evidence implicating epigenetic modifications as contributors to the altered gene expression after injury, with the potential to influence TBI outcomes.

Experimental studies focused on altering methylation after TBI have reported mixed reports. Existing literature suggests that dietary modification can be beneficial for the treatment of some neurological diseases [67-70]. Administering a ketogenic diet after TBI in adult animals reduced reactive neuroinflammation while also improving spatial memory performance [71]. These changes were linked to a significant increase in histone methylation and acetylation. In a study assessing the effects of selective SSRIs administration after TBI, the authors reported that fluoxetine treatment caused an increase in histone H3 acetylation in the hippocampus and DNA methylation transcription factor in the dentate gyrus [34]. Importantly, fluoxetine treatment did not result in any improved memory or locomotor function. Therefore, these rodent models of TBI shed light on changes of histone and DNA methylation, which are dependent on a specific subregion of the brain and on the post-injury time point. Ultimately, these studies showed a pro-inflammatory response and upregulation of protective factors targeted at repairing the TBI induced damage.

Conclusion

Epigenetics is a developing and important area of research, but its contribution and role in outcomes following head injuries is poorly understood. In this review, we have highlighted some recent understanding of the changes following neurotrauma in the context of epigenetics. These modifications may serve as key biomarkers and warrant further study. Improved understanding may also aid in enhanced therapeutics and novel treatment solutions that would lessen adverse clinical outcomes and promote neural repair.

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