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REVIEW

High mobility group box 1 (HMGB1) as a novel frontier in epileptogenesis: from pathogenesis to therapeutic approaches

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Abstract

Epilepsy is a serious neurological condition exhibiting complex pathology and deserving of more serious attention. More than 30% of people with epilepsy are not responsive to more than 20 anti-epileptic drugs currently available, reflecting an unmet clinical need for novel therapeutic strategies. Not much is known about the pathogenesis of epilepsy, but evidence indicates that neuroinflammation might contribute to the onset and progression of epilepsy following acquired brain insults. However, the molecular mechanisms underlying these pathophysiological processes are yet to be fully understood. The emerging research suggests that high-mobility group box protein 1 (HMGB1), a DNA-binding protein that is both actively secreted by inflammatory cells and released by necrotic cells, might contribute to the pathogenesis of epilepsy. HMGB1 as an initiator and amplifier of neuroinflammation, and its activation is implicated in the propagation of seizures in animal models. The current review will highlight the potential role of HMGB1 in the pathogenesis of epilepsy, and implications of HMGB1-targeted therapies against epilepsy. HMGB1 in this context is an emerging concept deserving further exploration. Increased understanding of HMGB1 in seizures and epilepsy will pave the way in designing novel and innovative therapeutic strategies that could modify the disease course or prevent its development.

Keywords: epilepsy, HMGB1, HMGB1 inhibitors, inflammation, RAGE, TLR4.

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Epilepsy is a serious neurological disorder characterized by the recurrent, periodic, and unpredictable occurrence of epileptic seizures which might be due to imbalance in excitatory and inhibitory pathways within the central nervous system (CNS) (Ana *et al.* 2019). Epilepsy has emerged as a serious health concern affecting 50–70 million people globally accounting 0.75% of global health burden (Trinka *et al.* 2018). Frequent and serious epileptic seizures are thought to contribute to further brain injury and persistent

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Abbreviations used: AEDs, anti-epileptic drugs; BBB, blood-brain-barrier; CA, cornu ammonis; CNS, central nervous system; CXCR4, C-X-C chemokine receptor type 4; DAMPs, damage associated molecular patterns; DRE, drug resistant epilepsy; EAE, experimental autoimmune encephalomyelitis; FCD, focal cortical dysplasia; FS, febrile seizure; HMGB1, high mobility group box 1; IHC, immunohistochemical staining; JAK-STAT, Janus kinases-signal transducer and activator of transcription proteins; KO, knock out; LPS-RS, lipopolysaccharide from rhodobacter sphaeroides; mAb, monoclonal antibodies; MAPK, mitogen-activated protein kinase; MES, maximal electro shock; NF-κB, nuclear Factor kappa-light-chain-enhancer of activated B cells; NMDAR, *N*-methyl-D-aspartate receptor; PG, prostaglandin; RAGE, receptor for advanced glycation end products; SE, status epilepticus; SRS, spontaneous recurrent seizures; TBI, traumatic brain injury; TIM-3, T-cell immunoglobulin and mucin domain-3; TLE, temporal lobe epilepsy; TLR-4, toll-like receptor 4; TNF, tumor necrosis factor; TREM-1, triggering receptor expressed on myeloid cells-1.

neurobehavioral and neuropsychiatric disorders, with significant consequences for patients, their families, and society (Yang *et al.* 2017). But much remains to be elucidated about the precise mechanisms of epileptogenesis – however, a plethora of findings over the past decade have highlighted the crucial pathophysiological role of brain inflammation in epilepsy (Vezzani *et al.* 2013; Webster *et al.* 2017; Paudel *et al.* 2018b).

The currently available anti-epileptic drugs (AEDs), which are not disease-modifying AEDs but rather anti-convulsants or anti-seizure drugs, provide only symptomatic relief. Further, the mainstream AEDs exert anti-epileptic effects largely by inhibiting voltage-gated Na^+, K^+, T-type Ca^{2+} channels (Simonato 2018), or by enhancing γ-aminobutyric acid signaling, and/or by inhibiting glutamate transmission to dampen the neuronal hyperexcitability (Mertens *et al.* 2018). But these AEDs are not the ultimate solution, with more than 30% of patients developing drug-resistant epilepsy (Chen *et al.* 2018), and increasing reports of adverse effects such as cognitive impairments, fatigue, and behavioral dysfunction in a significant proportion of patients treated with AEDs chronically (Sarkis *et al.* 2018). Despite recent advances in research, the etiology and the mechanisms of epileptogenesis still remain elusive, reinforcing the need for a deeper understanding of novel molecular targets. The ultimate goal of treatment is to prevent seizures with no side effects. Therefore, there is a pressing need for exploring novel treatment alternatives to minimize seizure burden as well as to prevent the related comorbidities. Currently, huge efforts have been made to understand the pathogenesis of epileptogenesis, and the pathways associated with epileptogenesis which exhibits great potentials in treating epilepsy. Considerable efforts ongoing in this regard include EPITARGET (Targets and Biomarkers for Anti-epileptogenesis) (EPITARGET 2018) and EpiBioS4Rx (Epilepsy Bioinformatics Study for Anti-Epileptogenic Therapy) (Vespa *et al.* 2018). EPITARGET is focusing mainly on identifying novel biomarkers and exploring the involvement of multiple mechanisms that might contribute to the process of epileptogenesis (EPITARGET 2018). Whereas EpiBioS4Rx mainly focused on anti-epileptogenesis in post-traumatic epilepsy following traumatic brain injury (TBI) (EpiBioS4Rx 2018). These both are large-scale consortium of researchers collecting information from patient populations, utilizing rigid and reliable standardized animal models, and cutting-edge analytical methods to analyze data from both animal and human for trans-species comparisons, which will help in the development of anti-epileptogenic therapies (EpiBioS4Rx 2018; EPITARGET 2018).

One of the emerging potential targets is high mobility group box 1 (HMGB1), and current understanding about the role of HMGB1 in epilepsy is rapidly growing. Moreover, the concept of understanding HMGB1 as a biomarker of epileptogenesis is also growing and has been well reported (Walker *et al.* 2016; Ravizza *et al.* 2017; Paudel *et al.* 2018a), which will aid in assessing disease progression and early prediction of disease onset. HMGB1 protein is known to contribute to epileptogenesis via a toll-like receptor (TLR) 4-dependent pathway to trigger tissue damage and inflammatory responses (Kleen and Holmes 2010). HMGB1 protein is a key initiator released by dying cells in the brain, and binds to TLR4 receptors and are abnormally released through neurons and astrocytes during an epileptic seizures (Maroso *et al.* 2010). The HMGB1-TLR4 regulatory axis is involved in the generation and exacerbation of epileptic seizures, and developing any innovative therapeutics which acts via inhibiting this signaling axis might represent a novel anti-epileptic treatment strategy. HMGB1 has emerged as a novel target against epileptogenesis, but much remained to be explored before translating it to patients (Fu *et al.* 2017). Herein, we highlight and update on key concepts ranging from HMGB1 biology, its role in epileptogenesis, available HMGB1 inhibitors and experimental evidence of HMGB1 as an anti-epileptic therapy. Based on available literature, it is postulated that HMGB1 is an emerging target against epilepsy and that pharmacological modulation of HMGB1 will provide disease-modifying effects.

Insights into HMGB1 biology

HMGB1 is an intriguing protein with complex biochemistry. It is a known danger signal or damage associated molecular pattern (Hei *et al.* 2018), and was first reported in 1973 as a non-histone chromosomal protein (Ranzato *et al.* 2010). Intracellular and extracellular HMGB1 protein has demonstrated several crucial roles in inflammation, including being implicated in sterile inflammation, immunity and neurodegenerative conditions, making it an attractive, and emerging target for therapeutic intervention (Andersson and Tracey 2011). Out of four members (HMGB1, HMGB2, HMGB3, and HMGB4) of the HMGB group of proteins (Štros 2010), HMGB1 is the only one that has been extensively studied with respect to epilepsy. The precise understanding of basic HMGB1 chemistry is crucial to understanding its function, as it is composed of different boxes with specific binding sites resulting in different activities. Structural analysis of HMGB1 revealed two DNA binding domains (box A and box B) and a negatively charged C-terminal (Ulloa and Messmer 2006; Yang *et al.* 2018). Box A contains the antagonistic site of box B, and demonstrates anti-inflammatory properties in both *in vivo* and *in vitro* models of inflammation (Girard 2007). In addition, box A alone serves as a competitive antagonist for HMGB1 and inhibits HMGB1 activity (Shen and Li, 2015). Box B is the functional domain which is recognized by TLR4, whereas both box B and box A can bind to DNA and play a role in folding and distorting the double-strand DNA (Dumitriu *et al.* 2005). The specific amino acid domains of HMGB1

such as 89–108 and 150–183 are responsible for binding to the TLR4 and receptor for advanced glycation end products (RAGE) respectively (Huttunen *et al.*, 2002). Moreover, the N-terminus contains heparin-binding motifs, whereas the box B exhibits pro-inflammatory activity to the protein and is involved in binding to RAGE (Huttunen and Rauvala 2004) (Fig. 1).

HMGB1 post-translational modification and receptor system

HMGB1 is reported to exist in three isoforms: fully reduced HMGB1, disulfide HMGB1, and sulfonyl HMGB1 (Antoine *et al.* 2014; Aucott *et al.* 2018a) (Table 1). Under normal conditions, HMGB1 is mainly located in the nucleus as a non-acetylated and thiol form whereas after tissue injury, non-acetylated thiol-HMGB1 is released from dead and dying cells, and can be further converted to disulfide-HMGB1 (Venereau *et al.* 2016). In addition, after cell activation or injury, translocation of HMGB1 from the nucleus to the cytoplasm may occur, followed by inflammasome activation and pyroptosis (Xu *et al.* 2014). Functional activity of HMGB1 is mainly determined by redox modification of 3 key cysteine residues, C23, C45, and C106. The disulfide and reduced isoforms of HMGB1 have mutually exclusive functions.

The fully reduced isoform of HMGB1 resides within the cell, and upon release, acts as a chemoattractant via complexation with CXCL12, binding exclusively through C-X-C chemokine receptor type 4 (Schiraldi *et al.* 2012). Based on its redox state, HMGB1 can stimulate cells through a range of receptors, including TLR, acting alone or in combination with other immune stimulants (Harris *et al.* 2012). Moreover, the reduced form of HMGB1 coordinates tissue regeneration whereas disulfide-HMGB1 triggers and sustains inflammation via the TLR4/myeloid differentiation factor-2 and RAGE receptors, but it is not involved in tissue regeneration (Tirone *et al.* 2018). HMGB1 also activates RAGE but the binding potency of each isoform for RAGE is yet to be known (Walker *et al.* 2017).

A diverse range of extracellular binding partners of HMGB1 has been reported and this includes RAGE, TLR9, TLR4, TLR2, integrin, synuclein filaments, proteoglycans, T-cell immunoglobulin and mucin domain, triggering receptor expressed on myeloid cells-1, cluster of differentiation 24 (CD24), C-X-C chemokine receptor type 4, and *N*-methyl-D-aspartate (NMDA) receptor (NMDAR) (Kang *et al.* 2014). However, RAGE and TLR4 are the two important binding

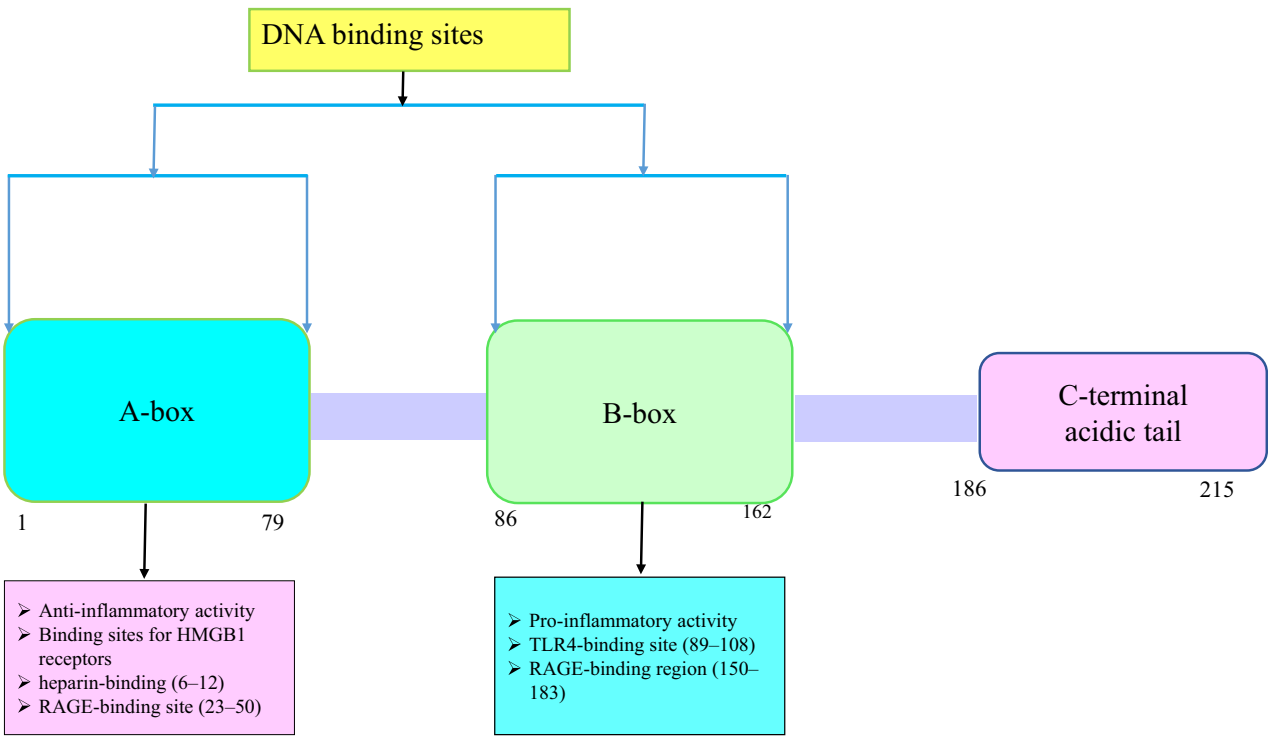
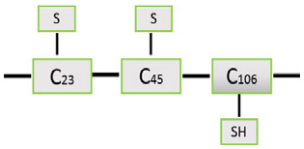
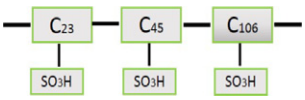


Fig. 1 Structural elucidation representing functionally relevant regions of HMGB1; modified from (Andersson *et al.* 2018a). HMGB1 protein is a chain of 215 amino acids with molecular weight of 25-kDa comprising of two DNA-binding domains, boxes A and B, and a negatively charged C-terminal tail. Box A acts as a specific antagonist of HMGB1 when isolated from the rest of the molecule (Bianchi and Manfredi 2007). HMGB1, high-mobility group box1; RAGE, receptor for advanced glycation end products; TLR4, Toll-like receptor 4.

Table 1 Description of redox HMGB1 with their biological activity

S.N.	Name	Schematic overview	Description	References
1.	Fully reduced (Fr) HMGB1		HMGB1 with no posttranslational modifications Thiol group is present on each of the cysteine residues Possess cell migration-inducing activity Lacks cytokine-inducing activity No HMGB1-TLR4 mediated cytokine production	Antoine *et al.* (2014), Andersson *et al.* (2018b) and Frigerio (2017)
2.	Disulfide (Ds) HMGB1		Contain disulphide bridge between C23 and C45 and a reduced C106 residues Is a TLR4 ligand Lacks cell migration-induced activity Possess cytokine-inducing activity	Aucott *et al.* (2018a) and Frigerio (2017)
3.	Sulfonyl (Ox) HMGB1		All the cysteine residues are terminally oxidized Lacks both cell migration-induced activity and cytokine-inducing activity No HMGB1-TLR4 mediated cytokine production	Aucott *et al.* (2018b) and Frigerio (2017)

HMGB1, high mobility group box 1; TLR4, Toll-like receptor.

partners that have been implicated in HMGB1 signaling as well as being studied extensively in epileptic conditions (Maroso *et al.* 2010, 2011). HMGB1 is predominantly fully reduced within the cell but can also be oxidized via reactive oxygen species upon translocation to the cytoplasm or post-extracellular release. Release pattern of HMGB1 from cells has been hypothesized via two distinct mechanisms: either from cells undergoing necrosis but not apoptosis, or secreted actively from cells after inflammatory cytokine stimulation (Tian *et al.* 2007). Upon its nuclear to cytoplasmatic translocation during seizures, following tissue injury, HMGB1 is extracellularly released and acts as a pro-inflammatory cytokine (Iori *et al.* 2013). The hyperacetylated form of HMGB1 is the one that regulates transcription of several pro-inflammatory cytokines, including IL-1β, via binding with TLR2, TLR4 as well as RAGE (Bianchi and Manfredi 2009; Maroso *et al.* 2011).

Binding of HMGB1 with RAGE and TLR4 elicits different responses. HMGB1/RAGE signaling regulates cell growth, proliferation, and migration via activation of mitogen-activated protein kinase and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways, whereas HMGB1/TLRs signaling mainly modulates inflammatory responses (Ding *et al.* 2017). RAGE is a pro-inflammatory receptor whereas TLR4 is the known HMGB1 receptor for cytokine production (Andersson *et al.* 2018a). HMGB1 demonstrates its biological activity by activating TLR4 receptor, but evidences which support the concept of a critical role of HMGB1 induced TLR4 activation in disease pathogenesis is lacking or not substantial (Branco-Madeira and Lambrecht 2010).

Mechanistic insights of HMGB1 in epileptogenesis: Insights from animal models

Inflammation-induced via several brain-disrupting events such as trauma, stroke, and infection are associated with epileptic seizures (Pitkänen and Sutula 2002). HMGB1 is one of the most influential pro-inflammatory cytokines and activates inflammatory pathways by stimulating two principal receptors: RAGE and TLR4 (Weber *et al.* 2015), and activation of these receptors have been implicated in epileptogenesis as depicted in Fig. 2. This process appears to be mediated by RAGE and TLR4, with evidence in particular of activation of HMGB1/TLR4 axis in experimental and human epilepsy and ictogenesis (Maroso *et al.* 2010).

In addition, increased levels of pro-inflammatory cytokines [IL-1β, tumor necrosis factor (TNF-α), HMGB1, S100β] and downstream inflammatory mediators (prostaglandins and the complement system) have been documented in epileptogenic tissues obtained from patients with different epilepsy etiologies (Aronica and Crino 2011; Vezzani and Friedman 2011). Moreover, the pro-inflammatory effect of HMGB1 is dependent on the activation of NF-κB or other pathways that promote chemotaxis and the production of cytokines (Li *et al.* 2016). NF-κB regulates immune and inflammatory responses and is also one of the crucial downstream transduction molecules in the TLR4 and RAGE signaling axis (Xie *et al.* 2013).

IL-1 is a family of pro-inflammatory cytokines and mediates innate immune response and IL-1β from the family is the one extensively investigated against epilepsy (Webster

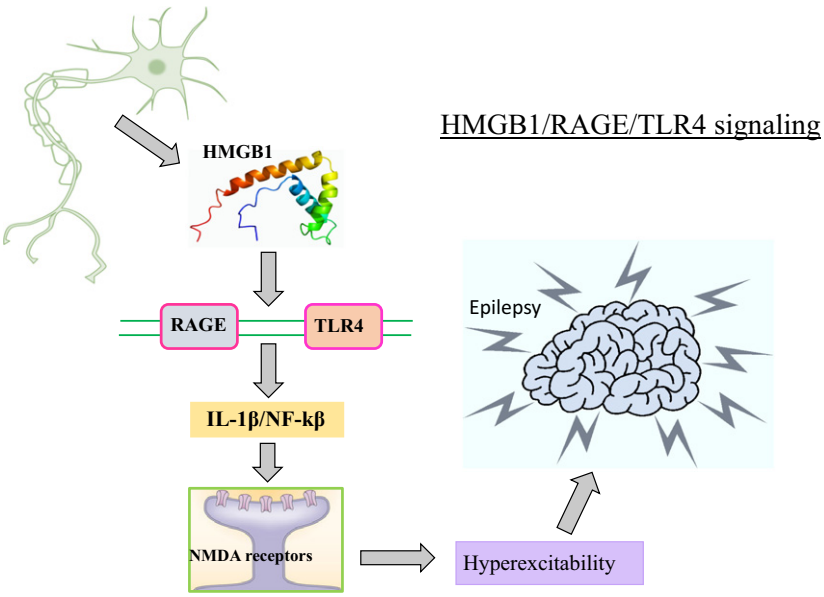


Fig. 2 HMGB1-TLR4 signaling in seizure generation (Paudel *et al.* 2018a). HMGB1 when released from glia and neuronal cells in the CNS activates its principal receptors (TLR4 and RAGE). Activation of HMGB1-TLR4 signaling axis leads to the phosphorylation of the NR2B subunit and the potentiation of NMDA-mediated Ca^{2+} influx into neurons. As well as, similar effects are also induced in neurons by IL-1B, released in contact with HMGB1 by glial cells during brain injury. Nevertheless, seizure generation and recurrence are dependent on activation of NMDA receptor (Andersson and Rauvala 2011; Balosso *et al.* 2014). HMGB1, high-mobility group box1; RAGE, receptor for advanced glycation end products; TLR4, Toll-like receptor 4.

et al. 2017). The impact of IL-1R/TLR signaling in epileptogenesis, characterized by neuronal hyperexcitability which might be provoked by IL-1β and HMGB1 provides insight into how IL-1R/TLR4 signaling might contribute in seizure generation after induction of inflammatory cascades (Iori *et al.* 2017). Activation of IL-1R1/TLR4 axis in receptor-expressing neurons promotes excitotoxicity and seizures through enhancing Ca^{2+} influx via NMDA receptors (Iori *et al.* 2013; Balosso *et al.* 2014) as well as might be a potential target for disease-modifying effects. Local CNS injury, or even a peripheral inflammatory challenge such as an infection, leads to the activation of microglia, astrocytes, and neurons in the brain. These cells then release pro-inflammatory cytokines such as IL-1β and HMGB1, that further initiate a sequence of inflammatory events in the target cells (neurons and glia) through activation of IL-1R1 and TLR4 (Vezzani *et al.* 2011). Transcriptional activation of inflammatory genes in glia might play a role in the perpetuation of brain inflammation which in turn results in the generation of individual seizures via lowering the threshold of neuronal excitability. This seizure exacerbation further activates inflammatory cascades via establishing feedback of events that contribute to the development of epilepsy (Maroso *et al.* 2011). A role for HMGB1 in mediating the activation of glial cells in epilepsy has also been unraveled, by mechanisms including the TLR4/NF-κB signaling axis. In a coriaria lactone (CL)-induced epilepsy model, CL was found to increase the levels of HMGB1, TLR4, RAGE, NF-κB p65, and inducible nitric oxide synthases in human microglia (HM) cells. Immunohistochemical staining suggested the nuclear to cytoplasmic translocation of HMGB1 in neurons and glial cells and reported its release into the extracellular space. HMGB1 contributes in epileptogenesis mainly through microglial activation, by the TLR4-NF-κB signaling axis activation (Shi *et al.* 2018). In a Picrotoxin/low Mg^{2+} entorhinal cortex (EC) slice model, IL-1β and HMGB1 favor ictal-like discharge production whereas in focal seizure model or 4-aminopyridine (4-AP) model it lowered ictal-like discharge threshold. In the 4-AP model, Ca^{2+} imaging experiments demonstrated that NMDA pulse applied to IL-1β and HMGB1-treated slices evoked a higher activation of neurons and astrocytes as compared to saline-treated slices. IL-1β or HMGB1 might decrease ictal frequency by elevating the sensitivity of neurons to NMDA thus causing a greater recruitment of neurons into the initial episode of NMDA receptor-mediated excitation in a local circuit. These results signify that IL-1β and HMGB1 can greatly exaggerate the generation of epileptiform activities (Chiavegato *et al.* 2014). Maroso and colleagues were the pioneers to shed light on the contribution of HMGB1 in epileptogenesis. The study elaborates the nature of HMGB1/TLR4 signaling, its intracellular signaling, and the contribution of HMGB1/TLR4 axis in kainic acid (KA) and bicuculline models of epileptogenesis. The elevated level of TLR4 and HMGB1 expression was observed in neurons, astrocytes, and microglia in the rat hippocampus as well as in human hippocampus from intractable temporal lobe epilepsy (TLE) patients (Maroso *et al.* 2010). Taken together, these findings suggest that TLR4 and, at a more downstream level, NMDA receptors, contribute to the pro-convulsant action of HMGB1 and suggest the plausible role of HMGB1-TLR4 signaling in the development and perpetuation of seizures. However, the precise mechanism behind the epileptogenesis remained enigmatic. Of note, mice lacking TLR4 or RAGE were less prone to developing epilepsy which suggests that HMGB1 is

implicated in epileptogenesis as well as ictogenesis in a TLR4-dependent way. Moreover, an antagonist of HMGB1 and TLR4 retard seizure precipitation and decrease recurrence of acute and chronic seizure (Maroso *et al.* 2010) implicating HMGB1-TLR4 axis as an emerging target against epileptic seizures. HMGB1 has gained increased attention after these findings, and numerous investigations focusing on the involvement of HMGB1 in epileptogenesis has been emerging in recent days. A recent study using intrahippocampal KA to induce a model of mesial TLE reported that the role of RAGE in seizures appears to be less prominent than that of TLR4. This statement was made on the basis of the significant decrease in KA seizures in RAGE knock out (KO) mice, whereas no delay in seizure onset was detected in TLR4 KO mice (Iori *et al.* 2013). Further, the proictogenic effect of exogenously-applied HMGB1 was reduced to a greater extent in RAGE KO compared to TLR4 KO mice.

One mechanism by which HMGB1 and other inflammatory mediators may exert pro-seizure effects may be via blood-brain-barrier (BBB) disruption. The BBB is important in establishing and maintaining the microenvironment of the CNS that permits proper neuronal function (Liebner *et al.* 2018). Several vasoactive or inflammatory compounds, which includes bradykinin, complement 3a, adenosine triphosphate (ATP), histamine and serotonin from mast cells, interleukins (IL), arachidonic acid and its metabolites, interferon alpha and beta, prostaglandins, and tumor necrosis factor (TNF), have all been reported to alter BBB permeability (Oby and Janigro 2006). The major players in the synthesis of these inflammatory compounds are brain-resident cells, namely activated microglia, astrocytes, and neurons (Devinsky *et al.* 2013). Invading peripherally-derived leukocytes are also posited to play a key role in epileptogenesis, especially when the BBB permeability is disrupted (Fabene *et al.* 2008). Pro-inflammatory mediators can induce and sustain BBB breakdown by modulating the endothelial tight junctions and the basal membrane as well as they might contribute in seizure activity by modulating excitability and seizure threshold in epileptic conditions (Abbott *et al.* 2006; Devinsky *et al.* 2013). It is not surprising that BBB permeability exhibits an important role in the pathogenesis of epilepsy (Oby and Janigro 2006). BBB disruption during epilepsy might allow HMGB1 and other inflammatory cytokines to intrude into the brain and aggravate a seizure. Disruption in BBB architecture can contribute to the development of epilepsy after status epilepticus (SE) through a direct mechanism (neuronal depolarization via the influx of potassium) or through a sequelae of events aggravated by leakage of serum proteins leading to activation of glial cells, impairment in potassium buffering, inflammation, and synaptogenesis (Gorter *et al.* 2015). After pro-convulsant (pilocarpine) administration in mice, a release of HMGB1 into the CNS and peripheral bloodstream was found to contribute to the induction of BBB disruption and activation of inflammatory mediators leading to epilepsy (Fu *et al.* 2017).

In spite of growing advances in our understanding of HMGB1, not much is known about its precise mechanism in the development of epileptogenesis. But there is a growing notion that HMGB1 might play role in the development of epileptogenesis, mainly disruption of BBB and induction of inflammatory processes. In an experimental model of pilocarpine-induced SE, there was a significant increment in the leakage of Evans Blue in the thalamus and hypothalamus regions indicating BBB disruption. Administration of recombinant human HMGB1 promoted Evans Blue leakage further in a dose-dependent fashion, which suggests that HMGB1 promotes the BBB breakdown in an epileptic state (Fu *et al.* 2017).

The putative role of HMGB1 isoforms in epilepsy is increasingly being established and has been well reviewed earlier (Ravizza *et al.* 2017). Recent evidence, however, also suggests an association between oxidative stress and neuroinflammation in the generation of the disulfide HMGB1 isoform. Precisely, oxidative stress during epileptogenesis is highly associated with *de novo* brain and blood generation of disulfide HMGB1 (Pauletti *et al.* 2017). In addition, the combination of anti-oxidant drugs (*N*-acetyl-cysteine and sulforaphane) when administered to epileptic rats reduced the onset of spontaneous seizures, inhibited disease progression and delayed seizure frequency, exerts neuroprotective effects and ameliorated cognitive impairments (Pauletti *et al.* 2017). These results implicate that the combinational drug inhibited the generation of disulfide HMGB1, via the reduction of oxidative stress, thus suggesting a potential novel therapeutic approach. Moreover, others have reported that the acetylated, disulfide form of HMGB1 is responsible for the detrimental inflammatory effects of epilepsy in animal models of epilepsy (Walker *et al.* 2016). HMGB1 in its oxidized (disulfide) form, most likely determined by the redox state of the extracellular milieu, potentiates NMDA-mediated calcium (Ca^{2+}) influx in pyramidal neuron cell bodies, by activation of neuronal TLR4 colocalized with NMDARs. HMGB1-TLR4 axis triggers neutral sphingomyelinase and Src kinases activities, and the phosphorylation of the NMDAR subunit 2B (Balosso *et al.* 2008). This rapid onset of post-translational pathway underlies HMGB1-NMDAR interactions, leading to intracellular Ca^{2+} increase and contributing to seizures and cell loss (Balosso *et al.* 2014). Necrotic cell death leads to the passive release of nonacetylated HMGB1. Disulfide HMGB1 binds and signals via TLR4 and induces pro-inflammatory and neuromodulatory effects through activation of NF-κβ (Yang *et al.* 2012).

A plausible role of HMGB1 in epilepsy of different etiologies is now advancing. Mesial TLE is known to be the common refractory focal epilepsy syndrome which usually emerges during childhood. Some of the key pathological characteristics including neuronal loss, axonal sprouting, and

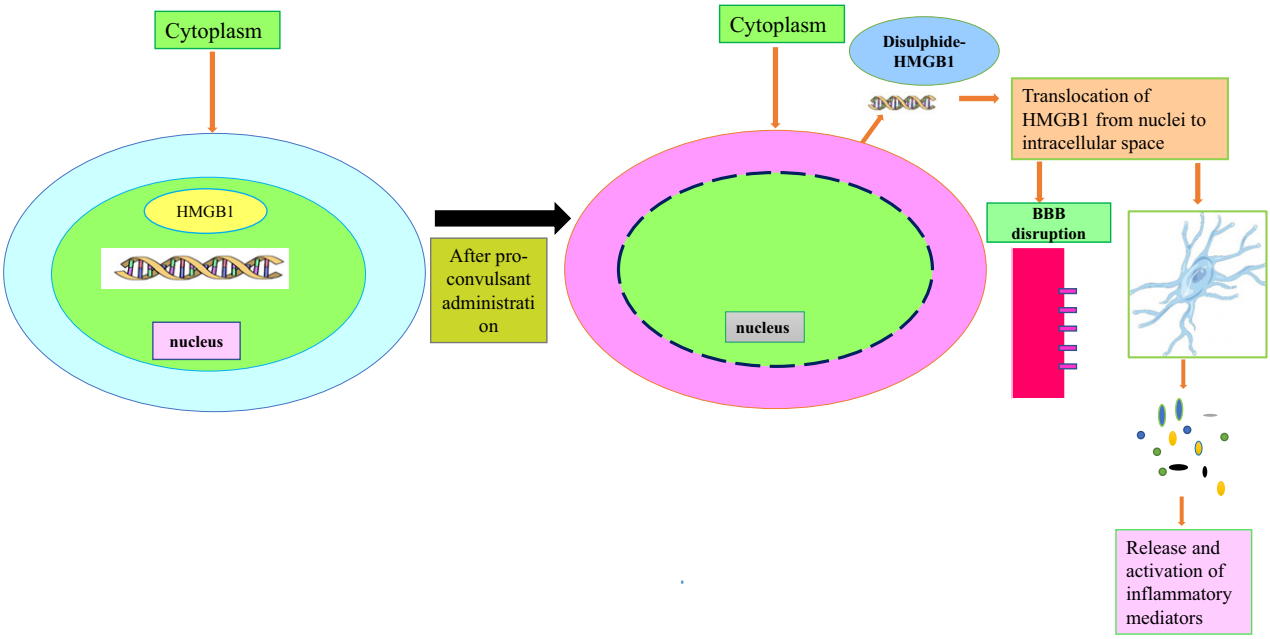


Fig. 3 HMGB1 translocation during epilepsy. After pro-convulsant administration there is a translocation of HMGB1 from nucleus to intracellular space and surrounding areas resulting in disruption of BBB and activation of inflammatory mediators. HMGB1, high-mobility group box1; BBB, blood-brain-barrier.

synaptic reorganization of hippocampal structures, which are also the pathological basis of refractory epilepsy (Toller *et al.* 2015). Activation of HMGB1/TLR4 signaling has been implicated in the pathogenesis of mesial TLE though the precise mechanism still remains elusive. In a pilocarpine-induced animal model of mesial TLE, quantitative real time-polymerase chain reaction (qRT-PCR) and western blot findings demonstrated a significant elevation of HMGB1 and TLR4 gene expression in the hippocampal tissues as compared to the control group. Moreover, enzyme-linked immunosorbent assay (ELISA) from neuron culture supernatants found that exogenously-applied HMGB1 increased IL-1β and TNF-α levels in hippocampal neurons (Yang *et al.* 2017). Focal cortical dysplasia (FCD) is one of the major causes of refractory epilepsy and is the most common histopathological type in children with lesional epilepsy (Blümcke *et al.* 2009). Western blot analysis revealed that HMGB1 protein expression in the cytoplasm and TLR4 protein expression in FCD lesion tissue from FCD type II patients was higher than that in peri-FCD tissue. MyD88 protein expression levels normalized to TLR4 in FCD lesions were higher than those in peri-FCD tissue. Moreover, the expression ratio of K63 polyubiquitin chain to TRAF6 was increased in FCD lesion tissue, compared to that in peri-FCD tissue. These findings suggested that that the HMGB1-TLR4 pathway was up-regulated in the neurons and astrocytes inside FCD type II lesions, and the up-regulation of HMGB1-TLR4 led to an increase in the release of downstream pro-inflammatory cytokines (Zhang *et al.* 2018). Febrile seizure (FS) is the most common form of childhood seizure occurring in 2–5% of children younger than 6 years (Kwon *et al.* 2018). Though HMGB1 has not been directly implicated in the pathogenesis of FS, elevated levels of serum HMGB1 along with cytokines (IL-1β, IL-6, IL-10, interferon-γ, and TNF-α) have been reported in patients (Choi *et al.* 2011). These findings implicate that HMGB1 and the cytokine network might also contribute to the generation of FS in children. Taken together, these findings support the observations made initially in experiment models, that HMGB1 and its receptors (RAGE and TLR4) levels are increased in epileptic tissue and may play a role in the development and perpetuation of epileptic seizures.

HMGB1 dynamics during epilepsy

Understanding the dynamics of HMGB1 translocation and activity is an important factor that will be helpful in assessing the disease pathology. Arrays of studies have repeatedly reported the release of HMGB1 from nuclei in a range of neurological disorders including epilepsy (Fu *et al.* 2017; Zhao *et al.* 2017; Angelopoulou *et al.* 2018; Sun *et al.* 2018). The nuclear to cytosol translocation of HMGB1 and its release from neural cells is associated with post-translational modifications and is implicated in the epileptic seizure generation (Fig. 3).

Current understanding of the dynamics of HMGB1 in epilepsy remains limited. Evidence of HMGB1 translocation after different pro-convulsant insults (KA, pentylenetetrazol,

Table 2 Summary of studies reporting HMGB1 translocation pattern after different pro-convulsant insult

S.N.	Model	Observation	Detection method	References
1	Pilocarpine-induced SE	HMGB1 translocation and release occurred in the CA1 and cerebral cortex regions ↓ HMGB1 levels in the brain and ↑ HMGB1 level in plasma in mice	Immunofluorescence staining, Western blot and ELISA	Fu *et al.* (2017
2	PTZ-induced seizure	HMGB1 was activated, translocated and released from nuclei in epileptic group HMGB1 was localized in the nuclei of neurons and astrocytes in control group	Immunohistochemical Staining	Zhao *et al.* (2017)
3	KA-induced epilepsy	HMGB1 was substantially increased in the pyramidal neurons in epileptic group HMGB1 immunoreactivity was detected mostly in the nuclei of the pyramidal neurons in control group	Immunohistochemical Staining	Chen *et al.* (2015)
4	KA-induced epilepsy	Expression level of HMGB1 in epileptic group was lower than the control group ($p < 0.05$) at 24 h, and became higher than the control group at 72 h ($p < 0.05$) which implicates a nucleus-to-cytoplasm translocation of HMGB1	Sombati's cell model (for *in vitro* study) and Immunohistochemistry	Huang *et al.* (2015)
5	KA-induced epilepsy	HMGB1 was induced in CA1 and CA3 hippocampal regions and peaked at 3 h and at 6 days post-KA administration	Immunoblot analysis	Luo *et al.* (2014)
6	eFSE	HMGB1 was localized to the nuclei of cells in control CA1 neurons HMGB1 appeared in the cytoplasm after 1and 3 h of eFSE, suggesting translocation of HMGB1 protein	Immunohistochemistry	Patterson *et al.* (2015)
7	KA-induced epilepsy	HMGB1 is present in the nuclei of pyramidal neurons, granule cells and dentate gyrus in control hippocampus Between 1 and 3 h of seizure onset progressive increase in nuclear and perinuclear HMGB1 staining observed in astrocytes	Immunohistochemical Staining	Maroso *et al.* (2010a)

↓, decreased; ↑, increased; SE, status epilepticus; PTZ, pentylenetetrazol; KA, kainic acid; HMGB1, high mobility group box protein 1; ELISA, enzyme linked immunosorbent assay; eFSE, experimental febrile status epilepticus; CA, cornu ammonis.

and Pilocarpine) are summarized in (Table 2) and elaborated in the text below. This translocated HMGB1 participates in the series of events leading to seizure generation with the interaction with RAGE (Zurolo *et al.* 2011) or TLR4 (Maroso *et al.* 2010).

The concept of HMGB1 post-translational modifications has been advanced in the context of epilepsy-related hyperexcitability. Immunoprecipitation (IP) of neurons exposed to KA excitotoxicity revealed that HMGB1 was acetylated, phosphorylated and ubiquitinated, implicating that post-translational modifications after KA administration caused HMGB1 to dissociate from chromatin DNA and promoted its release into the extracellular setting. Moreover, HMGB1 modulates the expression level of glutamate metabolism-associated enzymes in epilepsy-related hyper-excitability, postulating that HMGB1 signaling contributes in the generation of epileptic seizures (Kaneko *et al.* 2017). A similar line of evidence was reported which documented the expression level of HMGB1 in epileptic and control group at an interval of 24 and 72 h. In a KA-induced epilepsy model, the HMGB1 expression level in the epileptic group was lower as compared to control group ($p < 0.05$) at 24 h and increased compared to the control group at 72 h ($p < 0.05$). As well as *in vivo* analysis discovered a nucleus-to-cytoplasm translocation suggesting that HMGB1 is widely distributed in the cytoplasm in the epileptic group while HMGB1 is confined to the nucleus in control group (Huang *et al.* 2015).

Similarly, in an experimental febrile SE (eFSE) model, HMGB1 was localized inside the nuclei of cells in cornu ammonis (CA1) neurons of controls. After 1 and 3 h of eFSE, HMGB1-IR appeared in the cytoplasm, suggesting the translocation of HMGB1. Moreover, cytoplasmic HMGB1 was absent 24 h after eFSE and no differences in hippocampal HMGB1 mRNA levels were reported at any duration. Finally, mRNA expression of TLR4 was elevated significantly at 24 h after eFSE, and normalized to baseline levels 4 days after the eFSE. This finding implicates a transient translocation of hippocampal HMGB1 from the nucleus to the cytoplasm but not in microglia or astrocytes (Patterson *et al.* 2015). Immunoblot analysis suggest that HMGB1 was induced in the CA1 and CA3 hippocampi region in KA-induced model, and it peaked twice at 3 h and 6 days after-KA administration. The significant amount of HMGB1 was accumulated in serum at 12 h post-KA which might be due to HMGB1 release due to KA-induced neuronal death (Luo *et al.* 2014). Immunofluorescence staining study reveals that pilocarpine administration caused HMGB1 translocation from the nuclei, resulting in the low HMGB1 fluorescence intensity in the nuclei and an increased number of released HMGB1 particles. These

findings support the notion that there is HMGB1 translocation and release in the CA1 and cerebral cortex area during an acute epileptic state (Fu *et al.* 2017). In a KA-induced C57BL/6 mice, expression level and distribution of HMGB1 after KA induction suggest that HMGB1 immunoreactivity was mostly located inside the nuclei of the pyramidal neurons in the normal group. Moreover, in the stratum radiatum (SR), stratum lacunosum, and stratum moleculare, decentralized glial cells with nuclear staining and neurons with both nuclear and cytoplasmic staining were visualized. On the other side, cytoplasmic staining of HMGB1 was significantly elevated increased in the pyramidal neurons in an epileptic group. In addition to the glial cells with nuclear staining, glial cells with nuclear and cytoplasmic staining in SR, stratum lacunosum, and stratum moleculare of CA3 regions were reported. These findings corroborate to the nuclear to cytoplasmic translocation of HMGB1 in neurons and glial cells and postulated its release into the extracellular setting (Chen *et al.* 2015). Precise understanding on the dynamics of HMGB1 release and translocation after pro-convulsant insult will strengthen the knowledge in evaluating the therapeutic efficacy of HMGB1 inhibiting agent which probably inhibits the translocation of HMGB1 from nuclei into the neighbouring areas.

HMGB1-targeting therapeutic agents in epilepsy: An Update

Several strategies for the inhibition of HMGB1 have been explored. They range from HMGB1 antagonists capable of interacting with RAGE, small-molecule inhibitors of HMGB1, anti-HMGB1 antibodies (Ab), peptide and protein inhibitors of HMGB1, and oligonucleotide-based inhibitors of HMGB1 (Musumeci *et al.* 2014). Therapeutic strategies that target HMGB1 with specific Ab or antagonists have a potential for minimizing epileptic seizures characterized by excessive HMGB1 release. Nevertheless, the appreciable outcome has been achieved from experimental studies targeting extracellular HMGB1 only by anti-HMGB1 monoclonal antibodies (mAb) and HMGB1 inhibitors against epilepsy (Table 3). In addition, biological agents including anti-HMGB1 Ab, the recombinant A box peptide antagonist and glycyrrhizin (GL), a naturally-occurring HMGB1 antagonist derived from licorice root, are worthy of being explored against epilepsy as they can inhibit HMGB1 released by both activated macrophages and necrotic cells (Girard 2007).

Anti-HMGB1 mAb

Anti-HMGB1 mAb has emerged as a novel approach against several HMGB1 mediated pathologies (Liu *et al.* 2007; Okuma *et al.* 2012; Sasaki *et al.* 2016). To date, anti-HMGB1 Abs have been used mainly to confirm the contribution of HMGB1 in several pathological conditions, and the efficacy of its inhibition (Nishibori 2018). The therapeutical potential of anti-HMGB1 Abs has already been demonstrated in a range of HMGB1-mediated pathologies including experimental arthritis (Schierbeck *et al.* 2011), brain infarction (Liu *et al.* 2007), brain edema (Nosaka *et al.* 2018), and BBB permeability (Zhang *et al.* 2011). Nevertheless, very few studies to date have evaluated the potential role of anti-HMGB1 mAb against different models of epilepsy and reported noteworthy outcomes.

In a pilocarpine-induced seizure model, intravenous (i.v.) administration of anti-HMGB1 mAb exerts protective effects on neuronal apoptosis, in association with blockage of HMGB1 release, preventing the BBB disruption and inhibition of inflammation induced by pilocarpine (Fu *et al.* 2017). The reported anti-epileptic effect of anti-HMGB1 mAb might be due to the inhibition of BBB rupture, inflammatory responses, and neuronal cell death. Moreover, anti-HMGB1 mAb treatment was sufficient to suppress acute SE-induced translocation of HMGB1 into the peripheral setting (Fu *et al.* 2017). The overall result suggests that anti-HMGB1 mAb therapy might provide a novel strategy for preventing epileptogenesis.

The similar line of evidence was reported in another study incorporating both acute and chronic seizure models. Anti-HMGB1 mAb treatment attenuates maximal electroshock and pentylenetetrazol induced acute seizures, as well as inhibits the translocation and/or release of HMGB1 in astrocytes and neurons, as evidenced by demonstration of HMGB1 confined to cell nuclei (Zhao *et al.* 2017). But anti-HMGB1 mAb did not demonstrate any anti-seizure effect on TLR4 KO mice, which supports the notion that the HMGB1-TLR4 regulatory axis contributes to ictogenesis (Zhao *et al.* 2017). The anti-seizure effect of anti-HMGB1 mAb in the KA-induced seizure model and in the brain slices obtained from surgical resection of clinically drug-resistant human patients suggest that anti-HMGB1 mAb exhibits significant clinical therapeutic value for patients with medically refractory TLE (Zhao *et al.* 2017). A limited number of studies have also demonstrated the neuroprotective role of anti-HMGB1 Ab in neuronal damage and inflammation after SE. In a KA-induced model of SE, anti-HMGB1 Ab demonstrated dose-dependently inhibition of the mRNA expression of IL-1β and TNF-a, microglial activation, and neuronal damage implicating its neuroprotective potential. But this study did not report the anti-epileptic activity of anti-HMGB1 Ab (Li *et al.* 2013).

The therapeutic action of anti-HMGB1 mAb against epilepsy has been reported to initiate at 1 h and lasted until 24 h indicating that the mAb has a potential for clinical epilepsy treatment. Moreover, the anti-epileptic potential of anti-HMGB1 mAb on the human epileptic brain slices has not to be washed out implicating that anti-HMGB1 mAb binds solidly with HMGB1 solidly and has promising potentially long-term anti-epileptic activity (Zhao *et al.* 2017). In regards to the safety profile of anti-HMGB1 mAb, it only targets the activated, translocated HMGB1, and even in a high dose (25 mg/kg in mice) did not show any

Table 3 Summaries of studies reporting anti-epileptic effects of HMGB1 targeted therapies against experimental seizure models

S.N.	Interventions	Model	Treatment schedule	Observations	References
1	Anti-HMGB1 mAb	Pilocarpine-induced SE	Single bolus immediately after pilocarpine injection	Prevented the BBB permeability ↓ HMGB1 translocation Inhibited the expression of inflammation-related factors Protected against neural cell apoptosis Delay in latency and frequency of stage 5 seizure	Fu *et al.* (2017)
2	Anti-HMGB1 mAb	PTZ, KA and MES-induced seizure	Single bolus 3 h before PTZ 3 h post-SE for 7 days (KA) 1–48 h after bolus injection (MES)	Anti-HMGB1 mAb attenuates MES and PTZ-induced acute seizures as well as the translocation of HMGB1 Anti-HMGB1 alleviates KA induced chronic epilepsy Anti-HMGB1 mAb prevents epileptogenesis following SE	Zhao *et al.* (2017)
3	Box A (co-administered with IL-1Ra)	Electrical SE in rats	Single bolus 1 h post-SE onset, then daily injections for 7 days	↓ in progression of SRS frequency and ↓ number SRS	Walker *et al.* (2017)
4	Box A	KA and Bicucullineinduced seizure	Single bolus 15 min prior to KA or Bicuculline administration	↓ in seizure frequency and total duration; delay in seizure onset	Maroso *et al.* (2010a)
5	*N*-acethyl-cysteine and Sulforaphane.	Electrical SE in rats	Bolus injections 1–6 h post-SE onset then and then daily injections for 7–14 days	Prevented disulfide HMGB1 generation in brain and blood which is responsible for seizure generation ↓ in epilepsy onset, SRS and ↓ in SRS progression; Amelioration of cognitive deficits and exhibited neuroprotection	Pauletti *et al.* (2017)
6	LPS-RS	KA and Bicuculline induced seizure	Single bolus 15 min prior to KA or Bicuculline administration	↓ seizure number, duration as well as delay in seizure onset	Maroso *et al.* (2010a)
7	Glycyrrhizin (GL)	Pilocarpine induced SE	GL (50 mg/kg, i.p.) 30 min prior to pilocarpine administration	GL suppresses oxidative stress after SE GL attenuates expression of inflammatory markers after SE No anti-epileptogenic effects noted	González-Reyes *et al.* (2016)
8	GL	KA induced seizure	300 μL of GL 30 min prior KA injection	GL suppressed HMGB1 inductions in hippocampus GL exhibited anti-inflammatory and anti-excitotoxic functions	Luo *et al.* (2014)
9	GL	KA induced seizure	GL (10 mg/kg, i.p.) 30 min prior KA administration	GL inhibited KA-induced astrocyte and microglia activations in CA1 and CA3 hippocampal region GL suppressed KA-induced proinflammatory marker production GL demonstrated anti-excitotoxic effects in primary cortical cultures	Luo *et al.* (2013)
10	Celecoxib (CCX)	KA induced seizure	CCX (20 mg/kg) post-KA	CCX showed immunomodulatory effects on HMGB1-TLR4 pathway as evidenced by ↓ expression of HMGB1, TLR4 and COX-2 in cortex and hippocampus CCX ↓ frequency and severity of KA-induced seizure	Morales-Sosa *et al.* (2018)

↓, decreased; SE, status epilepticus; SRS, spontaneous recurrent seizure; PTZ, pentylenetetrazol; KA, kainic acid; MES, maximal electroshock; HMGB1, high mobility group box protein 1; mAb, monoclonal antibody; TLR4, toll-like receptor 4; GL, glycyrrhizin; BBB, blood-brain-barrier; Box A (competitive inhibitor of endogeneous HMGB1); LPS-RS, TLR4 antagonist (Lipopolysaccharide from Rhodobacter sphaeroides); IL-1Ra, interleukin-receptor antagonist; CA, cornu ammonis; CCX, celecoxib; COX-2, cyclooxygenase-2.

impairment in basic physical functions, body growth rate and thermoregulation (Zhao *et al.* 2017).

However, the Ab-based strategy to target HMGB1 is limited by issues such as the probability of conformational switches in the tertiary structure of the Ab-recognized region (Musumeci *et al.* 2014). In spite of that, anti-HMGB1 mAb demonstrated enough potential and specificity for therapy and has good drug-ability with wider therapeutic window, exhibiting enormous justification for the further development of this anti-HMGB1 mAb for the clinical management of epilepsy. Thus the development of human mAbs against HMGB1 and evaluation of their efficacy against several experimental epilepsy models should be the objectives of the future research.

HMGB1 inhibitors

A widely studied small-molecule inhibitor of HMGB1 is GL, investigated in a large number of HMGB1-mediated conditions, and reported to inhibit extracellular HMGB1 cytokine activity (Musumeci *et al.* 2014). GL (20β-carboxyl-11-oxo-30-norolean-12-en-3β-yl-2-*O*-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid) is the main bioactive component of *Glycyrrhiza radix*. GL is reported as a natural anti-inflammatory compound compared to an anti-HMGB1 mAb (Sun *et al.* 2018). Earlier studies have documented that GK has the potential to bind directly to both HMG boxes in HMGB1, thereby demonstrating its neuroprotective effects in a wide variety of neurological diseases (Gong *et al.* 2014). GL has exhibited protection against TBI via inhibition of HMGB1-RAGE interaction (Okuma *et al.* 2014), experimental autoimmune encephalomyelitis via inhibiting HMGB1 expression and neuronal HMGB1 release (Sun *et al.* 2018), and focal cerebral ischemia (Gong *et al.* 2014). Intracerebroventricular (i.c.v.) injection of KA-induced seizure in mice reported translocation of HMGB1 from the nuclei of neurons and astrocytes by 3 h post-KA injection. However, GL lowered the expression of HMGB1 in the hippocampus, and also decreased its release into serum after KA seizures (Luo *et al.* 2014). In a similar experiment, others have also reported that GL administration attenuates the KA-induced neuronal death in the CA1 and CA3 hippocampus region, which corresponds with decreases in the severity and duration of KA-induced seizures (Luo *et al.* 2013). In addition, GL protects rat hippocampus and olfactory bulb from oxidative and inflammatory damage induced by pilocarpine via inhibiting IL-1β and TNF-α in an experimental model of SE, although no anti-epileptic properties were reported in this study (González-Reyes *et al.* 2016). Recently, GL attenuated neuronal damage and altered disease progression post-SE by inhibiting HMGB1 activity and its translocation, and protected BBB integrity in an experimental SE model induced by lithium-pilocarpine (Li *et al.* 2018).

Surprisingly, the current state of knowledge on the anti-epileptic potential of GL via targeting HMGB1 in any seizure model is still lacking. Being a pharmacological inhibitor of HMGB1, GL has repeatedly demonstrated the suppression of HMGB1 expression in ranges of neurological disorders (Chen *et al.* 2017; Ieong *et al.* 2018). In this regard, GL deserves to be explored further in several acute and chronic seizure models.

Miscellaneous therapies targeting HMGB1

HMGB1 has been used as a target of interest in few studies, where the molecules have demonstrated anti-epileptic effects via inhibition of HMGB1. Box A (a competitive inhibitor of endogenous HMGB1), Ifenprodil (NR2B antagonist) and the TLR4 antagonist, lipopolysaccharide from *Rhodobacter sphaeroides* (LPS-RS), demonstrated their seizure retarding potential in a range of seizure models (Maroso *et al.* 2010). In spite of reported beneficial activities of box A in multiple experimental models, its mechanism of action has not been yet fully understood. Since box A is a fragment of HMGB1, the common understanding is that either it binds to HMGB1 receptors without activating them, or activating them very weakly (Venereau *et al.* 2016). Similarly, LPS-RS when administered 15 min before KA and Bicuculine in acute symptomatic seizures and spontaneous recurrent seizures (SRS) in mice models, delays seizure onset, seizure number, and total duration and delay SRS number and total duration respectively. Ifenprodil blocks HMGB1 and IL-1β and reduce the SRS number and duration in a model of SRS (Maroso *et al.* 2010). These results support the notion that HMGB1-TLR4 contributes to the seizure generation and severity. Therefore, the HMGB1 and TLR4 antagonist can effectively block seizure generation and seizure severity in a range of experimental models.

P-Glycoprotein (P-gp), known as an efflux transporter protein encoded by *multidrug resistance 1*, is a component of the BBB and reportedly highly associated with inflammation in the epileptic brain. HMGB1 is implicated in the upregulated expression of P-gp in the epileptic brain. In the KA-induced seizure model, HMGB1 up-regulated the expression level of P-gp, TLR4, and RAGE in bEnd.3 cells as detected by western blotting. Similarly, pre-treatment with Box A, LPS-RS, FPS-ZM1 (RAGE antagonist) and SN50 (NF-κB inhibitor) attenuated HMGB1-induced up-regulation of P-gp in bEnd. Three cells indicating that TLR4, RAGE, and NF-κB play important roles in HMGB1-induced overexpression of P-gp (Chen *et al.* 2015). This findings pave the way for future elucidation of the novel mechanisms underlying the overexpression of P-gp induced by seizure activities, and implicate that pharmacological intervention targeting HMGB1, TLR4, RAGE, and NF-κB could be effective against drug resistant epilepsy.

Micro RNA (MiRs) have also emerged as key players that regulates seizure-induced neuronal death and the innate immune response in the modulation of astrocyte-mediated inflammation (Liu *et al.* 2013). In an experimental model of autoimmune encephalomyelitis (AE)-related epilepsy, MiRs-

129-5p prevents the development of AE-related epilepsy via targeting HMGB1 expression and inhibiting TLR4/NF-kB signaling axis. Inhibition of HMGB1 by MiRs-129-5p has been suggested from luciferase reporter gene assay (Liu *et al.* 2017). These results pave the way in utilizing single-stranded RNAs that target HMGB1 which can provide a promising contribution in regards to the future treatment of epilepsy and related diseases.

Future perspective of HMGB1

The significance of targeting HMGB1 has been reported in several diseases, but considerable efforts are still required before it can be precisely targeted for any novel therapeutic strategies with clinical applicability (Venereau *et al.* 2016). Pharmacological modulation of the HMGB1-TLR/RAGE signaling axis with receptor antagonists or inactivating Ab might also represent a potentially novel anti-epileptic strategy. Developing a novel therapeutic approach for targeting HMGB1 requires a deeper understanding about its signaling pathways and the precise mechanisms involved in HMGB1 mediated disease pathology. Further research should be dedicated to creating more robust assays to evaluate functional bioactivity of HMGB1 antagonists (Andersson *et al.* 2018a). Moreover, a range of HMGB1 inhibitors such as ethyl pyruvate and salicylic acid should be evaluated for their therapeutic efficacy against epilepsy. In addition, future research should be in line with developing more powerful drugs that inhibit HMGB1 release, directly bind with HMGB1 and that impede the binding of HMGB1 to its receptors. Moreover, future HMGB1 antagonist should be designed in a way that it would selectively block to harmful disulfide isoform of HMGB1 overcoming the limitation of currently available HMGB1 antagonist which binds to all the three isoforms of HMGB1 (Andersson *et al.* 2018a). In addition it is equally important to investigate either one of the HMGB1 isoform dominates or regulates the activity of other isoforms (Gaskell *et al.* 2018) which is essential to elucidate the importance of HMGB1 in epileptogenesis and to design HMGB1 targeted therapies that specifically focus each HMGB1 isoform.

It is worth noting that in spite of the encouraging results demonstrated by HMGB1 inhibiting therapies against epilepsy in experimental studies, no appreciable clinical outcome has yet been reported. In order to translate the usability of HMGB1 as a target in a clinical setting, the development of a human monoclonal antibody (mAb) against HMGB1 is essential and further assessment of its efficacy not only against epilepsy, but also against epilepsy-induced cognitive dysfunction, is an unmet clinical need. Anti-HMGB1 mAb has demonstrated encouraging results and should be studied extensively as they have a disease modifying, long-term anti-epileptogenic effect, as evident by a reduced seizure frequency and improved cognitive functions in experimental studies (Zhao *et al.* 2017). However, developing novel therapeutic strategies that could regulate intracellular HMGB1 dynamics must still await a precise understanding of intracellular HMGB1 functions. Recent progress in understanding the fundamental biology of HMGB1 under physiological and pathological conditions will eventually provide headway towards developing anti-epileptic strategies targeting extracellular HMGB1.

On a pressing note, future investigations should focus to establish HMGB1 as a target for epilepsy and related comorbid conditions that share a bidirectional relationship with it; namely cognitive decline, depression, anxiety, autism, and schizophrenia (Paudel *et al.* 2018b). Among this array of comorbid conditions, cognitive dysfunction stands out as being highly associated with persons with epilepsy (PWE). Moreover, HMGB1 has been implicated in memory impairment by mediating the effects of RAGE and TLR4 (Mazarati *et al.* 2011). An earlier study alarmingly revealed that one half of PWE exhibit cognitive dysfunctions (Jensen 2011) in one or more domains, including learning, memory, attention, and executive functioning, though memory impairment is the most common (Allendorfer and Arida 2018). Currently, there is no anti-seizure drugs available with dual effects that can retard seizure frequency as well as improve associated cognitive dysfunction. Moreover, current AEDs are also reported to produce cognitive decline, in addition to its main role of minimizing seizures (Lagae 2006; Cavanna *et al.* 2010). This represents an immediate need to ameliorate the cognitive deficit experienced by PWE. Notably, there is a knowledge gap in terms of understanding the relationship between epilepsy and cognitive deficits, and that targeting the inflammatory response to prevent epileptogenesis (e.g. via HMGB1 inhibition) could add benefit of attenuating cognitive impairment. Surprisingly, in spite of its demonstrated role in epilepsy and cognitive dysfunctions, no study has reported the plausible role of HMGB1 in epilepsy induced cognitive decline. Hence, an immediate task would be to investigate the plausible role of the HMGB1 protein in epilepsy induced cognitive decline, which would further pave the way toward designing therapy that would improve the quality of life of PWE by ameliorating cognitive decline. Its worth noting that, recently anti-HMGB1 mAb prevented cognitive dysfunction induced by TBI implicating the role of HMGB1 in TBI induced cognitive decline (Okuma *et al.* 2018). In this regard, HMGB1 might be a suitable candidate to be modeled as a therapy that can retard the epileptic seizure, as well as ameliorate cognitive decline.

Challenges of HMGB1 as a therapy

The challenges not only lie in understanding the release pattern and downstream consequences of HMGB1 activity, but also extend toward the targeting of specific HMGB1 isoforms. To date, the role of HMGB1 redox forms in the context of epileptogenesis remains mostly unexplored, opening promising new avenues of investigation in this field. The

design and development of isoform-specific HMGB1 inhibitors might exert pharmacological control of inflammation-related neurological conditions like epilepsy (Venereau *et al.* 2016) though it has not been yet developed. Blocking disulfide HMGB1-activated cell signaling might mediate significant effects in several epilepsy models. As all-thiol isoform of HMGB1 demonstrated tissue protective role during inflammation, injury, and regeneration, it is challenging as well as desirable to design specific HMGB1 antagonists that do not simultaneously interfere with healing and the resolution of inflammation (Andersson *et al.* 2018a). Moreover, the chemical makeup of HMGB1 itself seems to be a challenge in developing HMGB1-based therapy as different isoforms behave differently (Agalave *et al.* 2015), as well as different boxes of HMGB1 protein itself possess different activity (Fig. 1 and Table 1). Different facets of HMGB1 such as its metabolism, stability, and biological activity of breakdown products generated from extracellular proteolytic cleavage are still intriguing. Though promising, HMGB1 as a therapy might be hindered due to several limiting factors such as safety issues, complexity of HMGB1 biology as well as its ability to interact with multiple signaling systems (Harris *et al.* 2012).

Moreover, the current findings regarding the expression level of HMGB1 in epileptic subjects is contradictory deserving further extensive investigations. A number of studies have reported an elevated expression level of HMGB1 in pilocarpine (Yang *et al.* 2017) and KA-induced seizure models in immature experimental animals (Morales-Sosa *et al.* 2018) as well as in clinical studies (Walker *et al.* 2014). On the contrary, decreased brain HMGB1 levels and increased plasma HMGB1 levels have also been reported in pilocarpine-treated mice (Fu *et al.* 2017) which lends credence to the idea that HMGB1 translocate from cell nuclei to the surrounding areas, including the bloodstream, after pilocarpine administration (Fu *et al.* 2017). Moreover, in a pilocarpine model of epilepsy, serum levels, but not brain levels of HMGB1 was elevated (Walker 2015). The discrepancies in studies on the levels of HMGB1 after pro-convulsant administration in different type of seizure models, leaves open the intriguing possibility that the nature of HMGB1 might be dependent on the seizure model, or severity, or brain region, and this warrants further investigation. Moreover, different notions of HMGB1 have also emerged regarding the role of HMGB1 in disease pathogenesis, but not purely on ongoing seizures (Walker *et al.* 2017). Though ranges of clinical and experimental findings reported HMGB1 is implicated in the pathogenesis if seizure disorders (Maroso *et al.* 2010) but either up-regulation occurred as a consequence of brain insult, seizures, epileptogenesis or the chronic epileptic state still remained poorly understood. It is possible that there are other, non-seizure related factors that are involved in the expression of HMGB1 during epilepsy (Walker 2015). This reflects the need to understand the relationship between seizure frequency and HMGB1 expression using different seizure model as well as an extensive investigation relating HMGB1 with epilepsy.

Summary and Conclusion

Current enthusiasm in exploring HMGB1 as an emerging target against epilepsy is increasing. The study of HMGB1 is a growing field with complex biology that deserves more attention. Progress in understanding the precise mechanisms underlying the pathogenic role of HMGB1 in epilepsy is instrumental for developing a treatment strategy with potential long-term disease-modifying effects by targeting HMGB1. Considering the role of HMGB1 in the pathogenesis of epilepsy, the identification of HMGB1 inhibitors and assessing their anti-epileptic effects could have significant experimental and clinical interest. The current review connects the dots between HMGB1 biology, its plausible contribution in the pathogenesis of epilepsy and available HMGB1 blocking therapies.

Herein, we have explored several insights into the contribution of HMGB1 in epilepsy and provided a perspective on the future development of novel treatments alternative targeting this extracellular protein, such as a specific antagonist against disulfide HMGB1. The concepts discussed herein provide compelling evidence that it is crucial to have a better understanding of the molecular and cellular changes underlying epilepsy and to identify the most promising HMGB1 based targets for anti-epileptic therapy. On a concluding note, the current review brings to light the concept of positing HMGB1 as a novel and emerging target against epileptogenesis. Future work elucidating the details of HMGB1 dynamics, structure, post-translational modification, and exploration of additional partners will undoubtedly unravel additional facets regarding HMGB1′s multiple functions (Kang *et al.* 2014).

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Authors' contribution

YNP and MFS carried out the literature review, conceptualized, designed and drafted the manuscript; BDS and NCJ contributed equally towards revising the manuscript and providing critical suggestion. IO contributed to the final manuscript. All authors read and approved the final manuscript.

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