MR. YAM NATH PAUDEL (Orcid ID : 0000-0003-0496-6526)

Article type      : Review

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

Yam Nath Paudel[1], Bridgette D. Semple[2,3$], Nigel C. Jones[2,3$], Iekhsan Othman[1] and Mohd. Farooq Shaikh[1*],

[1]Neuropharmacology Research Laboratory, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia (yam.paudel@monash.edu, farooq.shaikh@monash.edu and iekhsan.othman@monash.edu)

[2]Department of Neuroscience, Monash University, 99 Commercial Road, Melbourne, VIC, Australia

[3]Department of Medicine (Royal Melbourne Hospital), The University of Melbourne, Royal Parade, Parkville, VIC, Australia

(Bridgette.Semple@monash.edu and Nigel.jones@monash.edu)

$BDS and NCJ contributed equally to this paper

**\*Corresponding Author**

**Mohd. Farooq Shaikh, PhD**

Neuropharmacology Research Laboratory,

Jeffrey Cheah School of Medicine and Health Sciences,

MONASH University Malaysia,

Jalan Lagoon Selatan, Bandar Sunway,

Selangor, Malaysia.

Email: farooq.shaikh@monash.edu

Contact No: +603 5514 4483

**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 the more than 20 anti-epileptic drugs (AEDs) 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; RAGE; TLR4; Inflammation; HMGB1 inhibitors; seizure

**Abbreviation**

HMGB1= High Mobility Group Box 1

TLR-4 = Toll-Like Receptor 4

RAGE = Receptor for Advanced Glycation End Products

AEDs = Anti-Epileptic Drugs

SE= Status Epilepticus

TBI= Traumatic Brain Injury

BBB= Blood-Brain Barrier

TLE= Temporal Lobe Epilepsy

SRS= Spontaneous Recurrent Seizures

KO= Knock Out

DRE= Drug Resistant Epilepsy

MES= Maximal Electro Shock

MAPK= Mitogen-Activated Protein Kinase

NF-κB= Nuclear Factor kappa-light-chain-enhancer of activated B cells

CNS = Central Nervous System

DAMPs = Damage Associated Molecular Patterns

JAK-STAT = Janus Kinases- Signal Transducer and Activator of Transcription Proteins

NMDAR = N-methyl-D-Aspartate Receptor

TIM-3 = T-cell Immunoglobulin and Mucin Domain-3

TREM-1 = Triggering Receptor Expressed on Myeloid Cells-1

CXCR4 = C-X-C Chemokine Receptor Type 4

TNF= Tumor Necrosis Factor

IHC= Immunohistochemical Staining

FCD= Focal Cortical Dysplasia

FS= Febrile Seizure

CA= Cornu Ammonis

PG= Prostaglandin

mAb= Monoclonal Antibodies

EAE = Experimental Autoimmune Encephalomyelitis

LPS-RS= Lipopolysaccharide from Rhodobacter Sphaeroides

## 1. Introduction

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 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, Paudel *et al.* 2018b, Webster *et al.* 2017) .

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 (GABA) 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 (DRE) (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 (PTE) 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 analyse 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 (Ravizza *et al.* 2017, Paudel *et al.* 2018a, Walker *et al.* 2016), 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 & 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.* 2010a). 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.

## 2. Insights into HMGB1 biology

HMGB1 is an intriguing protein with complex biochemistry. It is a known danger signal or damage associated molecular pattern (DAMP) (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 & 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. Precise understanding of basic HMGB1 chemistry is crucial to understanding it's 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 & 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 & Rauvala 2004) (Figure1).
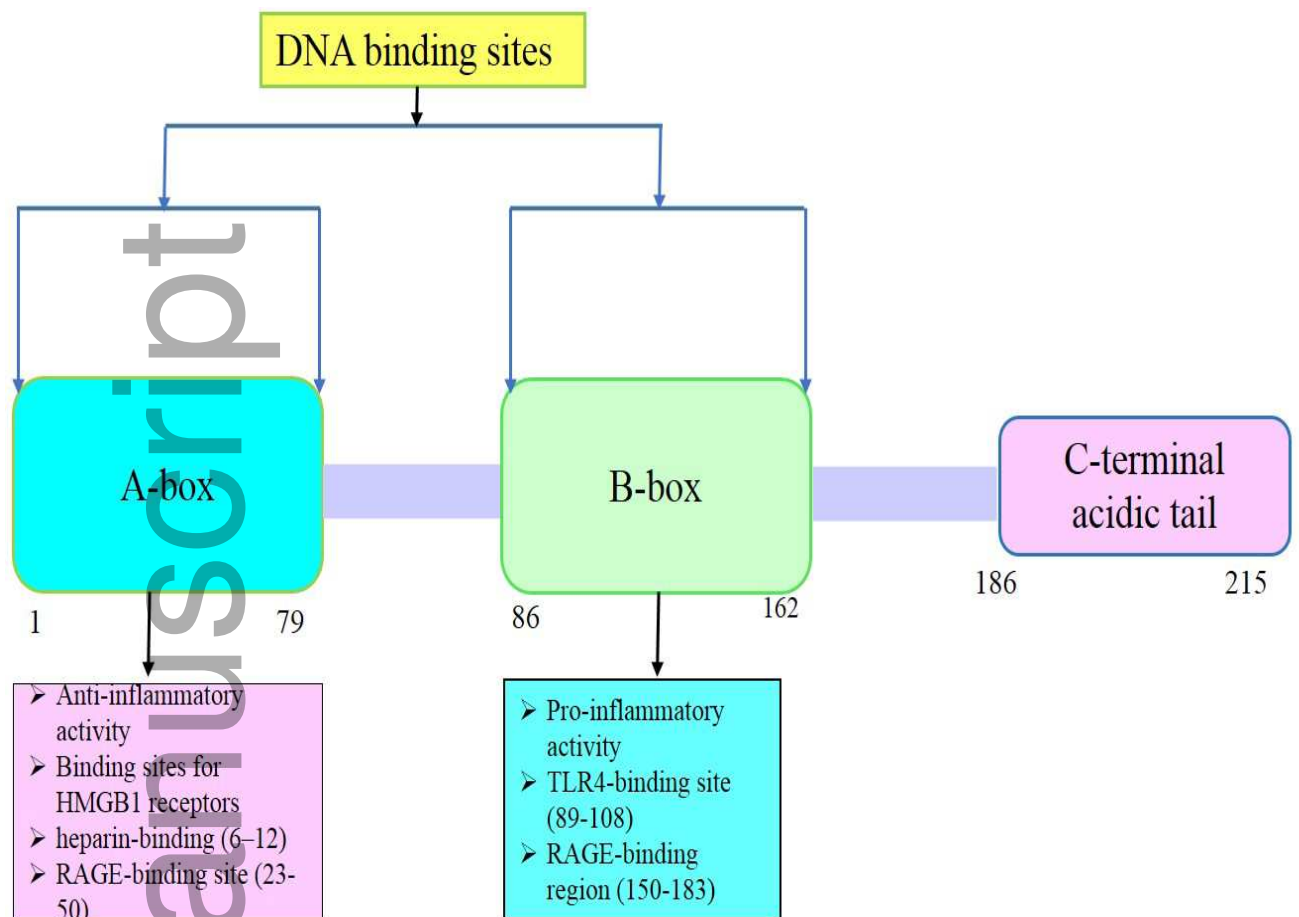
**Fig.1. Structural elucidation representing functionally relevant regions of HMGB1; modified from (Andersson *et al.* 2018a)**

*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 & Manfredi 2007). HMGB1, high-mobility group box1; RAGE, receptor for advanced glycation end products; TLR4, Toll-like receptor 4*

## 3. 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 CXCR4 (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 (MD-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 (TIM-3), triggering receptor expressed on myeloid cells-1 (TREM1), cluster of differentiation 24 (CD24), C-X-C chemokine receptor type 4 (CXCR4) and N-methyl-D-aspartate (NMDA) receptor (NMDAR) (Kang *et al.* 2014). However, RAGE and TLR4 are the two important binding partners that have been implicated in HMGB1 signaling as well as being studied extensively in epileptic conditions (Maroso et al. 2010a, Maroso *et al.* 2011). HMGB1 is predominantly fully reduced within the cell but can also be oxidized via reactive oxygen species (ROS) 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 & Manfredi 2009, Maroso et al. 2011).

Binding of HMGB1 with RAGE and TLR4 elicits different responses. HMGB1/RAGE signalling regulates cell growth, proliferation, and migration via activation of mitogen-activated protein kinase (MAPK) 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 & Lambrecht 2010).

## 4. 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 & 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 Figure 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. 2010a).
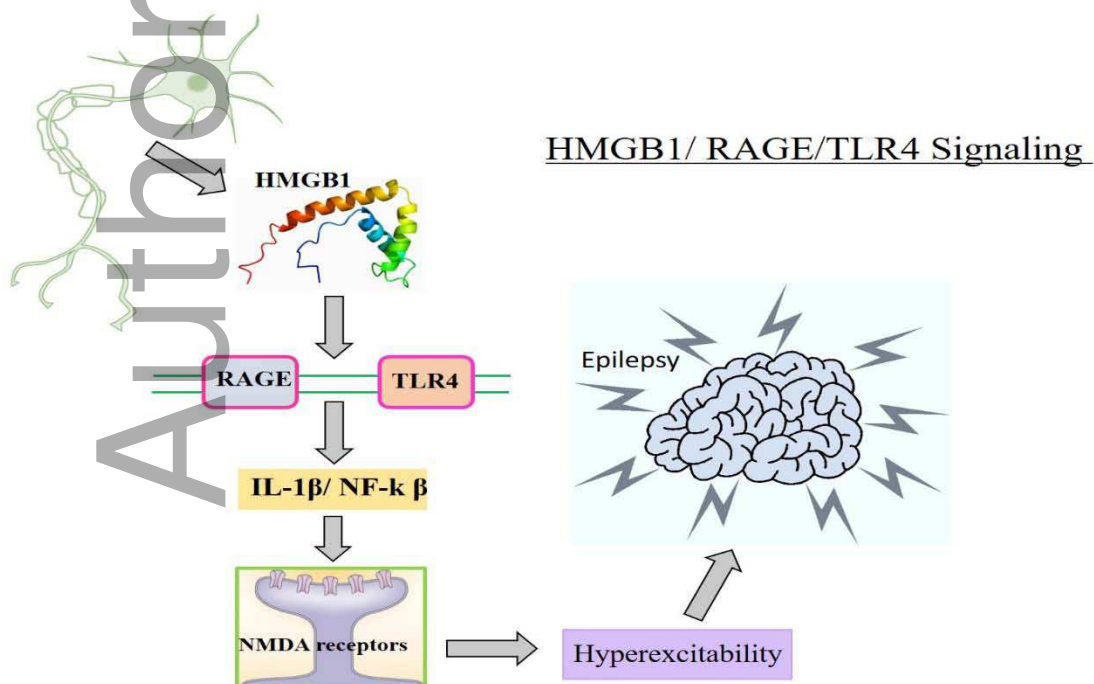
**Fig.2. HMGB1-TLR4 signaling in seizure generation (Paudel et al. 2018a)**

*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 & Rauvala 2011, Balosso et al. 2014).*

*HMGB1, high-mobility group box1; RAGE, receptor for advanced glycation end products; TLR4, Toll-like receptor 4*

In addition, increased levels of pro-inflammatory cytokines (IL-1β, 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 (Vezzani & Friedman 2011, Aronica & Crino 2011). Moreover, the pro-inflammatory effect of HMGB1 is dependant on 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 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 sequences 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 a 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 (iNOS) 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 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 result signifies 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 were 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.* 2010b). 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,

antagonist of HMGB1 and TLR4 retard seizure precipitation and decrease recurrence of acute and chronic seizure (Maroso et al. 2010a) implicating HMGB1-TLR4 axis as an emerging target against epileptic seizures . HMGB1 has gained increased attention after this 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 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 (IFN) alpha and beta, prostaglandins (PG), and tumor necrosis factor (TNF), have all been reported to alter BBB permeability (Oby & 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 (Devinsky et al. 2013, Abbott *et al.* 2006). It is not surprising that BBB permeability exhibits an important role in the pathogenesis of epilepsy (Oby & 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 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, 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 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 upregulated in the neurons and astrocytes inside FCD type II lesions, and the upregulation 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, IFN-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.

## 5. 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 nucleiin a range of neurological disorders including epilepsy (Sun *et al.* 2018, Angelopoulou *et al.* 2018, Fu et al. 2017, Zhao *et al.* 2017). 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 (Figure 3).
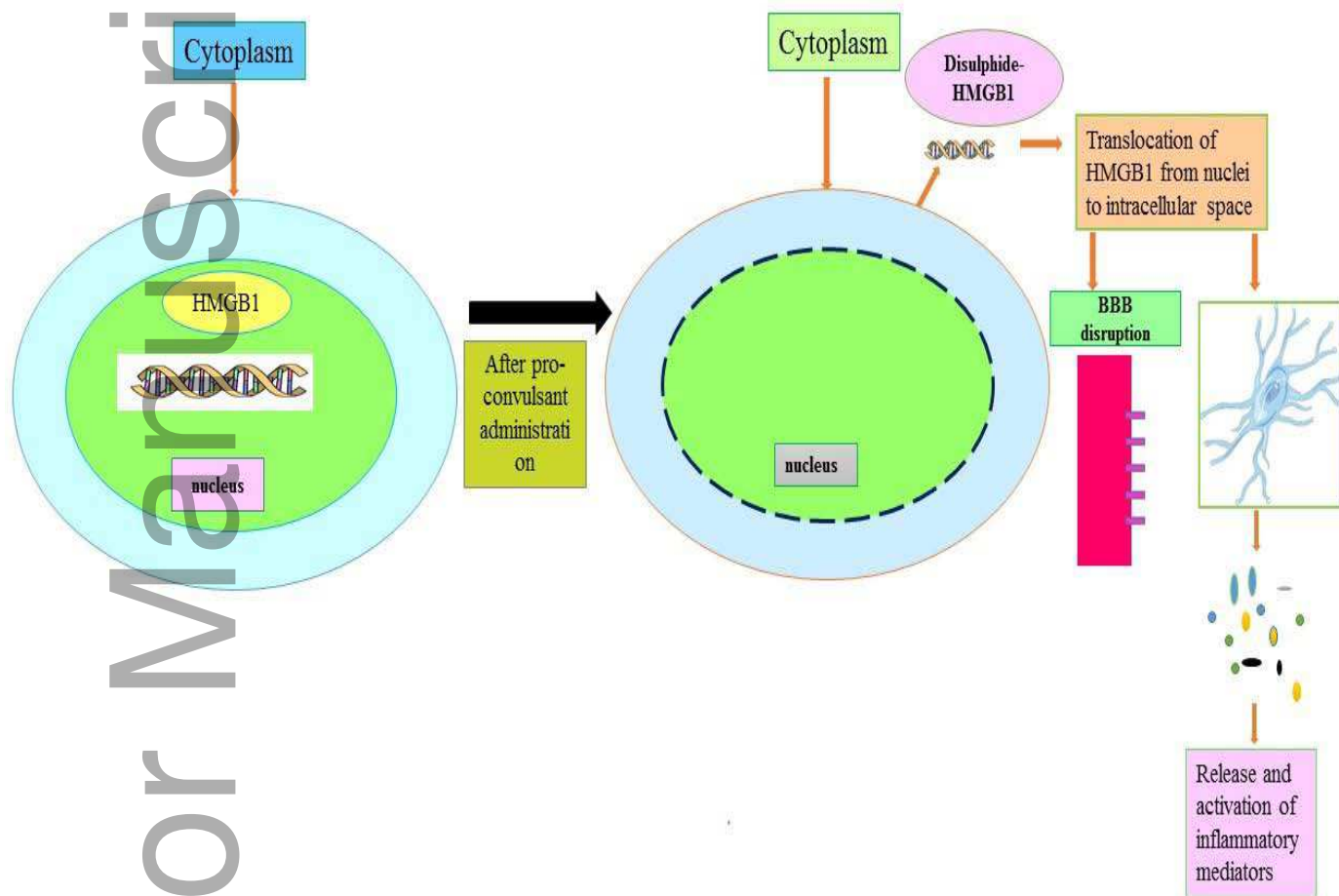


**Fig.3. HMGB1 translocation during epilepsy**

*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.*

Current understanding of the dynamics of HMGB1 in epilepsy remain limited. Evidence of HMGB1 translocation after different pro-convulsant insults (KA, PTZ, and Pilocarpine) are summarized in (Table 2) and elaborated in 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. 2010b).

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 hyperexcitability, postulating that HMGB1 signaling contributes in 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 hr. In a KA-induced epilepsy model, 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 durations. Finally, mRNA expression of TLR4 was elevated significantly at 24 h after eFSE, and normalized to baseline levels 4 d 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 (SL), and stratum moleculare (SM), 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, SL, and SM 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 neighboring areas.

## 6. 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).

### 6.1. Anti-HMGB1 mAb

Anti-HMGB1 mAb has emerged as a novel approach against several HMGB1 mediated pathologies (Sasaki *et al.* 2016, Liu *et al.* 2007, Okuma *et al.* 2012). 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 a 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 (MES) and pentylenetetrazol (PTZ) 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 a 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 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 have a potentials for clinical epilepsy treatment. Moreover, the anti-epileptic potential of anti-HMGB1 mAb on the human epileptic brain slices has not 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 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 enermous 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.

### 6.2. 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 GL has a 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 traumatic brain injury (TBI) via inhibition of HMGB1-RAGE interaction (Okuma *et al.* 2014), experimental autoimmune encephalomyelitis (EAE) 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, other 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 (Ieong *et al.* 2018, Chen *et al.* 2017). In this regard, GL deserves to be explored further in several acute and chronic seizure models.

### 6.3. 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. 2010b). 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 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. 2010a). 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 resistance1* (*MDR1*), 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 upregulated 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.3 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 DRE.

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.

## 7. 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 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 have 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 & 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 (Cavanna *et al.* 2010, Lagae 2006). 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 towards 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.

## 8. Challenges of HMGB1 as a therapy

The challenges not only lie in understanding the release pattern and downstream consequences of HMGB1 activity, but also extends towards 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 (Figure 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. 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. 2010a) 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 investigations relating HMGB1 with epilepsy.

## 9. 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).

**Conflict of Interest**

Authors declare that they have no potential conflict of interest.

| S.N. | Name | Schematic overview | Description | References |
|---|---|---|---|---|
| 1. | Fully reduced (Fr) HMGB1 | C$_{23}$ — C$_{45}$ — C$_{106}$ / SH SH SH | HMGB1 with no posttranslational modifications<br>Thiol group is present on each of the cysteine residues<br>Possess cell migration-inducing activity<br>Lacks cytokine-inducing activity<br>No HMGB1-TLR4 mediated cytokine production | (Antoine et al. 2014, Andersson *et al.* 2018b, Frigerio 2017) |
| 2. | Disulfide (Ds) HMGB1 | S S / C$_{23}$ — C$_{45}$ — C$_{106}$ / SH | Contain disulphide bridge between C23 and C45 and a reduced C106 residues<br>Is a TLR4 ligand<br>Lacks cell migration-induced activity<br>Possess cytokine-inducing activity | (Aucott et al. 2018a, Frigerio 2017) |

**Table.1. Description of redox HMGB1 with their biological activity**
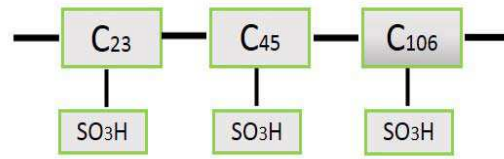
| 3. | Sulfonyl (Ox) HMGB1 | | All the cysteine residues are terminally oxidized | (Aucott *et al.* |
|---|---|---|---|---|
| | | | Lacks both cell migration-induced activity and cytokine-inducing activity | 2018b, Frigerio 2017) |
| | | | No HMGB1-TLR4 mediated cytokine production | |

$C_{23}$ — $C_{45}$ — $C_{106}$

$SO_3H$  $SO_3H$  $SO_3H$

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

**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<br><br>↓ 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<br><br>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<br><br>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 24h, and became higher than the control group at 72h ($p < 0.05$) 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<br>HMGB1 appeared in the cytoplasm after 1and 3 h of eFSE, suggesting translocation of HMGB1 protein | Immunohistoch emistry | (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<br>Between 1 and 3h of seizure onset progressive increase in nuclear and perinuclear HMGB1 staining observed in astrocytes | Immunohistoch emical Staining | (Maroso et al. 2010a) |

↓, decreased; SE, Status PTZ,

↑, increased; epilepticus;

Pentylenetetrazol; KA, Kainic acid; HMGB1, High mobility group box protein 1; ELISA, Enzyme linked immunosorbent assay; eFSE, Experimental febrile status epilepticus; CA, Cornu Ammonis; h, Hour;

| S.N. | Interventions | Model | Treatment schedule | Observations | References |
|------|------|-------|--------------------|--------------|-----------|
| 1 | Anti-HMGB1 | Pilocarpine-induced | Single bolus immediately after | prevented the BBB permeability ↓ HMGB1 translocation | (Fu et al. 2017) |

**Table 3.**

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

| | | | | | |
|---|---|---|---|---|---|
| | mAb | SE | pilocarpine injection | inhibited the expression of inflammation-related factors<br>protected against neural cell apoptosis<br>delay in latency and frequency of stage 5 seizure | |
| 2 | Anti-HMGB1 mAb | PTZ, KA and MES-induced seizure | Single bolus 3 h before PTZ<br>3 h post-SE for 7 days (KA)<br>1-48 h after bolus injection (MES) | Anti-HMGB1 mAb attenuates MES and PTZ-induced acute seizures as well as the translocation of HMGB1<br>Anti-HMGB1 alleviates KA induced chronic epilepsy<br>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 Bicuculline induced 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<br>GL suppressed KA-induced proinflammatory marker production<br>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<br>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; h, Hour;

Author Manuscript

## 10. References

Abbott, N. J., Rönnbäck, L. and Hansson, E. (2006) Astrocyte–endothelial interactions at the blood–brain barrier. *Nature Reviews Neuroscience,* **7,** 41.

Agalave, N., AbdelMoaty, S., Lundback, P., Andersson, U., Harris, H. and Svensson, C. (2015) Spinal disulfide HMGB1, but not all-thiol HMGB1, induces mechanical hypersensitivity in a TLR4-dependent manner. *Scandinavian journal of pain,* **8,** 47-47.

Allendorfer, J. B. and Arida, R. M. (2018) Role of physical activity and exercise in alleviating cognitive impairment in people with epilepsy. *Clinical therapeutics,* **40,** 26-34.

Ana, R., Mendes, M., Sousa, J., Pais, A., Falcão, A., Fortuna, A. and Vitorino, C. (2019) Rethinking carbamazepine oral delivery using polymer-lipid hybrid nanoparticles. *International journal of pharmaceutics,* **554,** 352-365.

Andersson, U. and Rauvala, H. (2011) Introduction: HMGB1 in inflammation and innate immunity. *Journal of internal medicine,* **270,** 296-300.

Andersson, U. and Tracey, K. J. (2011) HMGB1 is a therapeutic target for sterile inflammation and infection. *Annual review of immunology,* **29,** 139-162.

Andersson, U., Yang, H. and Harris, H. (2018a) Extracellular HMGB1 as a therapeutic target in inflammatory diseases. *Expert opinion on therapeutic targets,* **22,** 263-277.

Andersson, U., Yang, H. and Harris, H. (2018b) High-mobility group box 1 protein (HMGB1) operates as an alarmin outside as well as inside cells. In: *Seminars in immunology*. Elsevier.

Angelopoulou, E., Piperi, C. and Papavassiliou, A. G. (2018) High-mobility Group Box 1 in Parkinson's disease: from pathogenesis to therapeutic approaches. *Journal of neurochemistry*.

Antoine, D. J., Harris, H. E., Andersson, U., Tracey, K. J. and Bianchi, M. E. (2014) A systematic nomenclature for the redox states of high mobility group box (HMGB) proteins. *Molecular medicine,* **20,** 135.

Aronica, E. and Crino, P. B. (2011) Inflammation in epilepsy: clinical observations. *Epilepsia,* **52,** 26-32.

Aucott, H., Lundberg, J., Salo, H., Klevenvall, L., Damberg, P., Ottosson, L., Andersson, U., Holmin, S. and Harris, H. E. (2018a) Neuroinflammation in Response to Intracerebral Injections of Different HMGB1 Redox Isoforms. *Journal of innate immunity,* **10,** 215-227.

Aucott, H., Sowinska, A., Harris, H. E. and Lundback, P. (2018b) Ligation of free HMGB1 to TLR2 in the absence of ligand is negatively regulated by the C-terminal tail domain. *Molecular Medicine,* **24,** 19.

Balosso, S., Liu, J., Bianchi, M. E. and Vezzani, A. (2014) Disulfide-containing high mobility group box-1 promotes N-methyl-D-aspartate receptor function and excitotoxicity by activating Toll-like

receptor 4-dependent signaling in hippocampal neurons. *Antioxidants & redox signaling,* **21,** 1726-1740.

Balosso, S., Maroso, M., Sanchez-Alavez, M., Ravizza, T., Frasca, A., Bartfai, T. and Vezzani, A. (2008) A novel non-transcriptional pathway mediates the proconvulsive effects of interleukin-1β. *Brain,* **131,** 3256-3265.

Bianchi, M. E. and Manfredi, A. A. (2007) High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. *Immunological reviews,* **220,** 35-46.

Bianchi, M. E. and Manfredi, A. A. (2009) Dangers in and out. *Science,* **323,** 1683-1684.

Blümcke, I., Vinters, H. V., Armstrong, D., Aronica, E., Thom, M. and Spreafico, R. (2009) Malformations of cortical development and epilepsies. *Epileptic Disorders,* **11,** 181-193.

Branco-Madeira, F. and Lambrecht, B. N. (2010) High mobility group box-1 recognition: The beginning of a RAGEless era? *EMBO molecular medicine,* **2,** 193-195.

Cavanna, A. E., Ali, F., Rickards, H. E. and McCorry, D. (2010) Behavioral and cognitive effects of anti-epileptic drugs. *Discovery medicine,* **9,** 138-144.

Chen, X., Hua, H.-P., Liang, L. and Liu, L.-J. (2017) The Oral Pretreatment of Glycyrrhizin Prevents Surgery-Induced Cognitive Impairment in Aged Mice by Reducing Neuroinflammation and Alzheimer's-Related Pathology via HMGB1 Inhibition. *Journal of Molecular Neuroscience,* **63,** 385-395.

Chen, Y., Huang, X.-J., Yu, N., Xie, Y., Zhang, K., Wen, F., Liu, H. and Di, Q. (2015) HMGB1 contributes to the expression of P-glycoprotein in mouse epileptic brain through toll-like receptor 4 and receptor for advanced glycation end products. *PloS one,* **10,** e0140918.

Chen, Z., Brodie, M. J., Liew, D. and Kwan, P. (2018) Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: a 30-year longitudinal cohort study. *JAMA neurology,* **75,** 279-286.

Chiavegato, A., Zurolo, E., Losi, G., Aronica, E. and Carmignoto, G. (2014) The inflammatory molecules IL-1β and HMGB1 can rapidly enhance focal seizure generation in a brain slice model of temporal lobe epilepsy. *Frontiers in cellular neuroscience,* **8,** 155.

Choi, J., Min, H. J. and Shin, J.-S. (2011) Increased levels of HMGB1 and pro-inflammatory cytokines in children with febrile seizures. *Journal of neuroinflammation,* **8,** 135.

Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N. C. and Rogawski, M. A. (2013) Glia and epilepsy: excitability and inflammation. *Trends in neurosciences,* **36,** 174-184.

Ding, J., Cui, X. and Liu, Q. (2017) Emerging role of HMGB1 in lung diseases: friend or foe. *Journal of cellular and molecular medicine,* **21,** 1046-1057.

Dumitriu, I. E., Baruah, P., Manfredi, A. A., Bianchi, M. E. and Rovere-Querini, P. (2005) HMGB1: guiding immunity from within. *Trends in immunology,* **26,** 381-387.

EpiBioS4Rx (2018) Epilepsy Bioinformatics Study for Antiepileptogenic Therapy.

EPITARGET (2018) EPITARGET - Targets and biomarkers for antiepileptogenesis. Vol. 2018. EPITARGET.

Fabene, P. F., Mora, G. N., Martinello, M. et al. (2008) A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nature medicine,* **14,** 1377.

Frigerio, F. (2017) Identifying Key Regulators of the Immune/Inflammatory Response for Developing Anticonvulsive and Antiepileptogenic Approaches. The Open University.

Fu, L., Liu, K., Wake, H., Teshigawara, K., Yoshino, T., Takahashi, H., Mori, S. and Nishibori, M. (2017) Therapeutic effects of anti-HMGB1 monoclonal antibody on pilocarpine-induced status epilepticus in mice. *Scientific Reports,* **7,** 1179.

Gaskell, H., Ge, X. and Nieto, N. (2018) High-Mobility Group Box-1 and Liver Disease. *Hepatology communications,* **2,** 1005-1020.

Girard, J.-P. (2007) A direct inhibitor of HMGB1 cytokine. *Chemistry & biology,* **14,** 345-347.

Gong, G., Xiang, L., Yuan, L., Hu, L., Wu, W., Cai, L., Yin, L. and Dong, H. (2014) Protective effect of glycyrrhizin, a direct HMGB1 inhibitor, on focal cerebral ischemia/reperfusion-induced inflammation, oxidative stress, and apoptosis in rats. *PLoS One,* **9,** e89450.

González-Reyes, S., Santillán-Cigales, J. J., Jiménez-Osorio, A. S., Pedraza-Chaverri, J. and Guevara-Guzmán, R. (2016) Glycyrrhizin ameliorates oxidative stress and inflammation in hippocampus and olfactory bulb in lithium/pilocarpine-induced status epilepticus in rats. *Epilepsy research,* **126,** 126-133.

Gorter, J. A., van Vliet, E. A. and Aronica, E. (2015) Status epilepticus, blood–brain barrier disruption, inflammation, and epileptogenesis. *Epilepsy & Behavior,* **49,** 13-16.

Harris, H. E., Andersson, U. and Pisetsky, D. S. (2012) HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nature Reviews Rheumatology,* **8,** 195.

Hei, Y., Chen, R., Yi, X., Long, Q., Gao, D. and Liu, W. (2018) HMGB1 Neutralization Attenuates Hippocampal Neuronal Death and Cognitive Impairment in Rats with Chronic Cerebral Hypoperfusion via Suppressing Inflammatory Responses and Oxidative Stress. *Neuroscience,* **383,** 150-159.

Huang, J., Wu, Y., Huang, Q., Li, S., Ye, J., Wei, X., Liu, Q., Liu, Y. and Ma, M. (2015) Expression level and distribution of HMGB1 in Sombati's cell model and kainic acid-induced epilepsy model. *Eur Rev Med Pharmacol Sci,* **19,** 2928-2933.

Huttunen, H. and Rauvala, H. (2004) Amphoterin as an extracellular regulator of cell motility: from discovery to disease. *Journal of internal medicine,* **255,** 351-366.

Ieong, C., Sun, H., Wang, Q. and Ma, J. (2018) Glycyrrhizin suppresses the expressions of HMGB1 and ameliorates inflammative effect after acute subarachnoid hemorrhage in rat model. *Journal of Clinical Neuroscience,* **47,** 278-284.

Iori, V., Iyer, A. M., Ravizza, T. et al. (2017) Blockade of the IL-1R1/TLR4 pathway mediates disease-modification therapeutic effects in a model of acquired epilepsy. *Neurobiology of disease,* **99,** 12-23.

Iori, V., Maroso, M., Rizzi, M. et al. (2013) Receptor for Advanced Glycation Endproducts is upregulated in temporal lobe epilepsy and contributes to experimental seizures. *Neurobiology of disease,* **58,** 102-114.

Jensen, F. E. (2011) Epilepsy as a spectrum disorder: implications from novel clinical and basic neuroscience. *Epilepsia,* **52,** 1-6.

Kaneko, Y., Pappas, C., Malapira, T., Vale, F. Ĺ., Tajiri, N. and Borlongan, C. V. (2017) Extracellular HMGB1 Modulates Glutamate Metabolism Associated with Kainic Acid-Induced Epilepsy-Like Hyperactivity in Primary Rat Neural Cells. *Cellular Physiology and Biochemistry,* **41,** 947-959.

Kang, R., Chen, R., Zhang, Q. et al. (2014) HMGB1 in health and disease. *Molecular aspects of medicine,* **40,** 1-116.

Kleen, J. K. and Holmes, G. L. (2010) Taming TLR4 may ease seizures. *Nature medicine,* **16,** 369.

Kwon, A., Kwak, B. O., Kim, K., Ha, J., Kim, S.-J., Bae, S. H., Son, J. S., Kim, S.-N. and Lee, R. (2018) Cytokine levels in febrile seizure patients: A systematic review and meta-analysis. *Seizure,* **59,** 5-10.

Lagae, L. (2006) Cognitive side effects of anti-epileptic drugs: the relevance in childhood epilepsy. *Seizure,* **15,** 235-241.

Li, G., Wu, X., Yang, L., He, Y., Liu, Y., Jin, X. and Yuan, H. (2016) TLR4-mediated NF-κB signaling pathway mediates HMGB1-induced pancreatic injury in mice with severe acute pancreatitis. *International journal of molecular medicine,* **37,** 99-107.

Li, Y. j., Wang, L., Zhang, B., Gao, F. and Yang, C. M. (2018) Glycyrrhizin, an HMGB1 inhibitor, exhibits neuroprotective effects in rats after lithium-pilocarpine-induced status epilepticus. *Journal of Pharmacy and Pharmacology*.

Li, Z., Li, B., Zhu, X., Yin, P., Liu, J., Huang, S. and Sun, R. (2013) Neuroprotective effects of anti-high-mobility group box 1 antibody in juvenile rat hippocampus after kainic acid-induced status epilepticus. *Neuroreport,* **24,** 785-790.

Liebner, S., Dijkhuizen, R. M., Reiss, Y., Plate, K. H., Agalliu, D. and Constantin, G. (2018) Functional morphology of the blood–brain barrier in health and disease. *Acta neuropathologica*, 1-26.

Liu, A.-H., Wu, Y.-T. and Wang, Y.-P. (2017) MicroRNA-129-5p inhibits the development of autoimmune encephalomyelitis-related epilepsy by targeting HMGB1 through the TLR4/NF-kB signaling pathway. *Brain research bulletin, 132,* 139-149.

Liu, H., Roy, M. and Tian, F.-F. (2013) MicroRNA-based therapy: a new dimension in epilepsy treatment. *International Journal of Neuroscience, 123,* 617-622.

Liu, K., Mori, S., Takahashi, H. K. et al. (2007) Anti-high mobility group box 1 monoclonal antibody ameliorates brain infarction induced by transient ischemia in rats. *The FASEB Journal, 21,* 3904-3916.

Luo, L., Jin, Y., Kim, I.-D. and Lee, J.-K. (2013) Glycyrrhizin attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. *Experimental neurobiology, 22,* 107-115.

Luo, L., Jin, Y., Kim, I.-D. and Lee, J.-K. (2014) Glycyrrhizin suppresses HMGB1 inductions in the hippocampus and subsequent accumulation in serum of a kainic acid-induced seizure mouse model. *Cellular and molecular neurobiology, 34,* 987-997.

Maroso, M., Balosso, S., Ravizza, T. et al. (2010a) Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nature medicine, 16,* 413.

Maroso, M., Balosso, S., Ravizza, T. et al. (2010b) Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nature Medicine, 16,* 413.

Maroso, M., Balosso, S., Ravizza, T., Liu, J., Bianchi, M. and Vezzani, A. (2011) Interleukin-1 type 1 receptor/Toll-like receptor signalling in epilepsy: the importance of IL-1beta and high-mobility group box 1. *Journal of internal medicine, 270,* 319-326.

Mazarati, A., Maroso, M., Iori, V., Vezzani, A. and Carli, M. (2011) High-mobility group box-1 impairs memory in mice through both toll-like receptor 4 and receptor for advanced glycation end products. *Experimental neurology, 232,* 143-148.

Mertens, L. J., Witt, J.-A. and Helmstaedter, C. (2018) Affective and behavioral dysfunction under antiepileptic drugs in epilepsy: Development of a new drug-sensitive screening tool. *Epilepsy & Behavior, 83,* 175-180.

Morales-Sosa, M., Orozco-Suárez, S., Vega-García, A., Caballero-Chacón, S. and Feria-Romero, I. A. (2018) Immunomodulatory effect of Celecoxib on HMGB1/TLR4 pathway in a recurrent seizures model in immature rats. *Pharmacology Biochemistry and Behavior, 170,* 79-86.

Musumeci, D., Roviello, G. N. and Montesarchio, D. (2014) An overview on HMGB1 inhibitors as potential therapeutic agents in HMGB1-related pathologies. *Pharmacology & therapeutics, 141,* 347-357.

Nishibori, M. (2018) HMGB1 as a representative DAMP and anti-HMGB1 antibody therapy. *Nihon yakurigaku zasshi. Folia pharmacologica Japonica,* **151,** 4-8.

Nosaka, N., Hatayama, K., Yamada, M., Fujii, Y., Yashiro, M., Wake, H., Tsukahara, H., Nishibori, M. and Morishima, T. (2018) Anti-high mobility group box-1 monoclonal antibody treatment of brain edema induced by influenza infection and lipopolysaccharide. *Journal of medical virology,* **90,** 1192-1198.

Oby, E. and Janigro, D. (2006) The blood–brain barrier and epilepsy. *Epilepsia,* **47,** 1761-1774.

Okuma, Y., Liu, K., Wake, H. et al. (2014) Glycyrrhizin inhibits traumatic brain injury by reducing HMGB1–RAGE interaction. *Neuropharmacology,* **85,** 18-26.

Okuma, Y., Liu, K., Wake, H. et al. (2012) Anti–high mobility group box-1 antibody therapy for traumatic brain injury. *Annals of neurology,* **72,** 373-384.

Okuma, Y., Wake, H., Teshigawara, K. et al. (2018) Anti-High Mobility Group Box-1 Antibody Therapy May Prevent Cognitive Dysfunction after Traumatic Brain Injury. *World neurosurgery*.

Patterson, K. P., Brennan, G. P., Curran, M., Kinney-Lang, E., Dubé, C., Rashid, F., Ly, C., Obenaus, A. and Baram, T. Z. (2015) Rapid, coordinate inflammatory responses after experimental febrile status epilepticus: implications for epileptogenesis. *Eneuro*, ENEURO. 0034-0015.2015.

Paudel, Y. N., Shaikh, M., Serrano, Á. A., Kumari, Y., Aleksovska, K., Alvim, M. K. M., Chakraborti, A. and Othman, I. B. (2018a) HMGB1: A Common Biomarker and Potential Target for TBI, Neuroinflammation, Epilepsy and Cognitive Dysfunction. *Frontiers in Neuroscience,* **12,** 628.

Paudel, Y. N., Shaikh, M. F., Shah, S., Kumari, Y. and Othman, I. (2018b) Role of inflammation in epilepsy and neurobehavioral comorbidities: Implication for therapy. *European journal of pharmacology*.

Pauletti, A., Terrone, G., Shekh-Ahmad, T. et al. (2017) Targeting oxidative stress improves disease outcomes in a rat model of acquired epilepsy. *Brain,* **140,** 1885-1899.

Pitkänen, A. and Sutula, T. P. (2002) Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *The Lancet Neurology,* **1,** 173-181.

Ranzato, E., Patrone, M., Pedrazzi, M. and Burlando, B. (2010) Hmgb1 promotes wound healing of 3T3 mouse fibroblasts via RAGE-dependent ERK1/2 activation. *Cell biochemistry and biophysics,* **57,** 9-17.

Ravizza, T., Terrone, G., Salamone, A., Frigerio, F., Balosso, S., Antoine, D. J. and Vezzani, A. (2017) High mobility group box 1 is a novel pathogenic factor and a mechanistic biomarker for epilepsy. *Brain, behavior, and immunity*.

Sarkis, R. A., Goksen, Y., Mu, Y., Rosner, B. and Lee, J. W. (2018) Cognitive and fatigue side effects of anti-epileptic drugs: an analysis of phase III add-on trials. *Journal of neurology*, 1-6.

Sasaki, T., Liu, K., Agari, T. et al. (2016) Anti-high mobility group box 1 antibody exerts neuroprotection in a rat model of Parkinson's disease. *Experimental neurology,* **275,** 220-231.

Schierbeck, H., Lundbäck, P., Palmblad, K., Klevenvall, L., Erlandsson-Harris, H., Andersson, U. and Ottosson, L. (2011) Monoclonal anti-HMGB1 (high mobility group box chromosomal protein 1) antibody protection in two experimental arthritis models. *Molecular medicine,* **17,** 1039.

Schiraldi, M., Raucci, A., Muñoz, L. M. et al. (2012) HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. *Journal of experimental medicine,* **209,** 551-563.

Shi, Y., Zhang, L., Teng, J. and Miao, W. (2018) HMGB1 mediates microglia activation via the TLR4/NF-κB pathway in coriaria lactone induced epilepsy. *Molecular medicine reports,* **17,** 5125-5131.

Simonato, M. (2018) Epilepsy an Update on Disease Mechanisms: The Potential Role of MicroRNAs. *Frontiers in neurology,* **9,** 176.

Štros, M. (2010) HMGB proteins: interactions with DNA and chromatin. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms,* **1799,** 101-113.

Sun, Y., Chen, H., Dai, J., Wan, Z., Xiong, P., Xu, Y., Chai, W., Gong, F. and Zheng, F. (2018) Glycyrrhizin Protects Mice against Experimental Autoimmune Encephalomyelitis by Inhibiting HMGB1 Expression and Neuronal HMGB1 Release. *Frontiers in immunology,* **9,** 1518.

Tian, J., Avalos, A. M., Mao, S.-Y. et al. (2007) Toll-like receptor 9–dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nature immunology,* **8,** 487.

Tirone, M., Tran, N. L., Ceriotti, C. et al. (2018) High mobility group box 1 orchestrates tissue regeneration via CXCR4. *Journal of Experimental Medicine,* **215,** 303-318.

Toller, G., Adhimoolam, B., Rankin, K. P., Huppertz, H.-J., Kurthen, M. and Jokeit, H. (2015) Right fronto-limbic atrophy is associated with reduced empathy in refractory unilateral mesial temporal lobe epilepsy. *Neuropsychologia,* **78,** 80-87.

Trinka, E., Kwan, P., Lee, B. and Dash, A. (2018) Epilepsy in Asia: Disease burden, management barriers, and challenges. *Epilepsia*.

Ulloa, L. and Messmer, D. (2006) High-mobility group box 1 (HMGB1) protein: friend and foe. *Cytokine & growth factor reviews,* **17,** 189-201.

Venereau, E., De Leo, F., Mezzapelle, R., Careccia, G., Musco, G. and Bianchi, M. E. (2016) HMGB1 as biomarker and drug target. *Pharmacological research,* **111,** 534-544.

Vespa, P. M., Shrestha, V., Abend, N. et al. (2018) The epilepsy bioinformatics study for anti-epileptogenic therapy (EpiBioS4Rx) clinical biomarker: Study design and protocol. *Neurobiology of disease*.

Vezzani, A., Aronica, E., Mazarati, A. and Pittman, Q. J. (2013) Epilepsy and brain inflammation. *Experimental neurology,* **244,** 11-21.

Vezzani, A. and Friedman, A. (2011) Brain inflammation as a biomarker in epilepsy. *Biomarkers in medicine,* **5,** 607-614.

Vezzani, A., Maroso, M., Balosso, S., Sanchez, M.-A. and Bartfai, T. (2011) IL-1 receptor/Toll-like receptor signaling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures. *Brain, behavior, and immunity,* **25,** 1281-1289.

Walker, L., Tse, K., Ricci, E., Thippeswamy, T., Sills, G. J., White, S. H., Antoine, D. J., Marson, A. and Pirmohamed, M. (2014) High mobility group box 1 in the inflammatory pathogenesis of epilepsy: profiling circulating levels after experimental and clinical seizures. *The Lancet,* **383,** S105.

Walker, L. E. (2015) Defining the mechanistic role of High-Mobility Group Box-1 and its Utility as a Biomarker in the Inflammatory Pathogenesis of Epilepsy. University of Liverpool.

Walker, L. E., Frigerio, F., Ravizza, T. et al. (2017) Molecular isoforms of high-mobility group box 1 are mechanistic biomarkers for epilepsy. *The Journal of clinical investigation,* **127,** 2118-2132.

Walker, L. E., Janigro, D., Heinemann, U., Riikonen, R., Bernard, C. and Patel, M. (2016) WONOEP appraisal: molecular and cellular biomarkers for epilepsy. *Epilepsia,* **57,** 1354-1362.

Weber, D. J., Allette, Y. M., Wilkes, D. S. and White, F. A. (2015) The HMGB1-RAGE inflammatory pathway: implications for brain injury-induced pulmonary dysfunction. *Antioxidants & redox signaling,* **23,** 1316-1328.

Webster, K. M., Sun, M., Crack, P., O'Brien, T. J., Shultz, S. R. and Semple, B. D. (2017) Inflammation in epileptogenesis after traumatic brain injury. *Journal of neuroinflammation,* **14,** 10.

Xie, J., Méndez, J. D., Méndez-Valenzuela, V. and Aguilar-Hernández, M. M. (2013) Cellular signalling of the receptor for advanced glycation end products (RAGE). *Cellular signalling,* **25,** 2185-2197.

Xu, J., Jiang, Y., Wang, J. et al. (2014) Macrophage endocytosis of high-mobility group box 1 triggers pyroptosis. *Cell death and differentiation,* **21,** 1229.

Yang, H., Lundbäck, P., Ottosson, L. et al. (2012) Redox modification of cysteine residues regulates the cytokine activity of high mobility group box-1 (HMGB1). *Molecular medicine,* **18,** 250.

Yang, L., Wang, F., Yang, L., Yuan, Y., Chen, Y., Zhang, G. and Fan, Z. (2018) HMGB1 a-Box Reverses Brain Edema and Deterioration of Neurological Function in a Traumatic Brain Injury Mouse Model. *Cellular Physiology and Biochemistry,* **46,** 2532-2542.

Yang, W., Li, J., Shang, Y., Zhao, L., Wang, M., Shi, J. and Li, S. (2017) HMGB1-TLR4 axis plays a regulatory role in the pathogenesis of mesial temporal lobe epilepsy in immature rat model and children via the p38MAPK signaling pathway. *Neurochemical research,* **42,** 1179-1190.

Zhang, J., Takahashi, H. K., Liu, K. et al. (2011) Anti-high mobility group box-1 monoclonal antibody protects the blood–brain barrier from ischemia-induced disruption in rats. *Stroke,* **42,** 1420-1428.

Zhang, Z., Liu, Q., Liu, M. et al. (2018) Upregulation of HMGB1-TLR4 inflammatory pathway in focal cortical dysplasia type II. *Journal of neuroinflammation,* **15,** 27.

Zhao, J., Wang, Y., Xu, C. et al. (2017) Therapeutic potential of an anti-high mobility group box-1 monoclonal antibody in epilepsy. *Brain, behavior, and immunity,* **64,** 308-319.

Zurolo, E., Iyer, A., Maroso, M. et al. (2011) Activation of Toll-like receptor, RAGE and HMGB1 signalling in malformations of cortical development. *Brain,* **134,** 1015-1032.
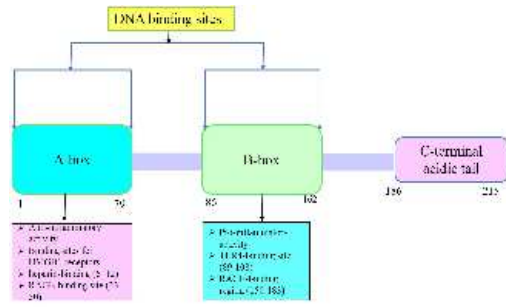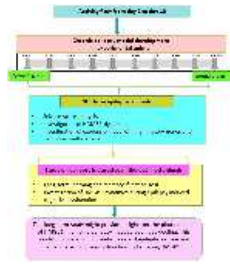
jnc_14663_f1.jpg
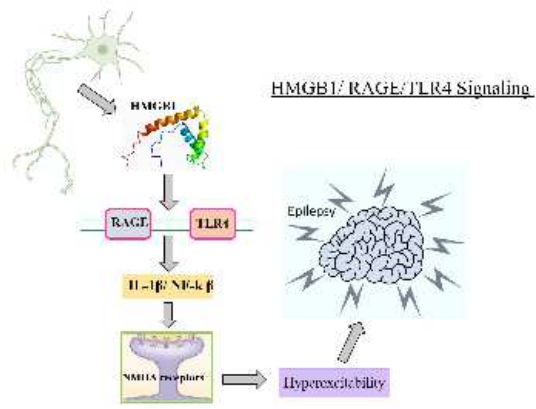
jnc_14663_f2.jpg
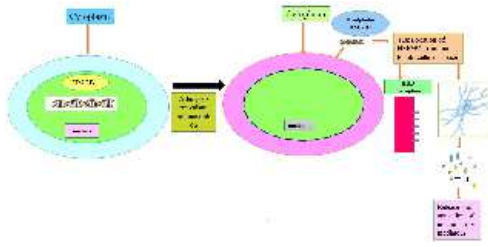
jnc_14663_f3.jpg

jnc_14663_f4.jpg

Author/s:
Paudel, YN; Semple, BD; Jones, NC; Othman, I; Shaikh, MF

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

Date:
2019-12-01

Citation:
Paudel, Y. N., Semple, B. D., Jones, N. C., Othman, I. & Shaikh, M. F. (2019). High mobility group box 1 (HMGB1) as a novel frontier in epileptogenesis: from pathogenesis to therapeutic approaches. JOURNAL OF NEUROCHEMISTRY, 151 (5), pp.542-557. https://doi.org/10.1111/jnc.14663.

Persistent Link:
http://hdl.handle.net/11343/285441

File Description:
Accepted version