Understanding epileptogenesis from molecules to network alteration

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Epilepsy is characterized by recurrent seizures. Following an initial insult, a latent period precedes the onset of spontaneous seizures, a process referred to as epileptogenesis. This period plays a critical role in halting the progression toward epilepsy before the onset of abnormal molecular and network alterations. In this study, the fundamental concepts of epileptogenesis as well as the associated molecular and cellular targets are reviewed.

Keywords: Epilepsy, Physiopathology, Biomarkers

Introduction

Epilepsy is characterized by recurrent seizures and is a highly burdensome disease, ranking fifth in disability-adjusted life years among neurologic diseases [1]. Epileptogenesis is the gradual process by which an initially undamaged, ‘healthy’ brain becomes susceptible to seizures. Despite years of extensive epilepsy research aimed at decoding the crucial molecular mechanisms and identifying targets for novel drug development, discovering a drug to inhibit epileptogenesis remains elusive. This review elucidates the fundamental concepts of epileptogenesis from an educational perspective, offering a detailed spatiotemporal understanding of the key structural and molecular biological changes involved. In addition, it discusses diagnostic and therapeutic biomarkers to further enhance understanding in this field.

Understanding the experimental model of epilepsy

A goal of epilepsy research is to identify molecular targets that can halt or mitigate epileptogenesis to prevent the onset of epilepsy. To achieve this objective, several acute seizure models and chronic epilepsy animal models have been developed. Chemical models use substances such as pilocarpine, kainic acid, bicuculline, and pentylentetrazol, which are administered either directly or systemically, inducing seizures immediately after injection. Target molecules have been evaluated both before and simultaneously with stimuli, and their effects have been evaluated based on reduction or delay of seizure onset. The use of electric or hyperthermic stimuli follows a similar concept, albeit with different methods of stimulation. Although these methods may demonstrate an antiseizure effect, they do not necessarily indicate an antiepileptogenic effect. To verify antiepileptogenic effects, it is necessary to understand the mechanism of spontaneous seizures.
Following acute injury from chemicals and the acute seizure event, tissue progressively becomes more susceptible to seizures, ultimately leading to spontaneous seizures after a certain time interval known as the latent period. The duration of the latent period varies depending on the developmental stage, the nature of the injury, and the size of the animal used. For instance, the brains of rodents may take a few weeks to exhibit spontaneous seizures after treatment with kainic acid, whereas traumatic injury in humans can take several years to develop into posttraumatic epilepsy [2-4]. Understanding this timeline is crucial for designing experiments.

Despite experimental successes in antiepileptogenesis, an antiepileptogenic drug has not yet achieved clinical success. This discrepancy may be attributed to differences between humans and animals as well as issues inherent in the experimental process. For example, in many experiments, an epileptogenic effect is caused by administering a drug for a certain period during the latent phase following an initial stimulus and then counting the number and incidence of spontaneous seizures and the number of animals developing epilepsy. However, the possibility of insufficient drug clearance from the brain raises concerns about the validity of these findings.

Another significant aspect is the nonuniform occurrence of seizures that tend to cluster [5,6], indicating that the frequency of seizures over a short period may not accurately reflect the efficacy of a drug but rather the natural cycle of the condition. To overcome this, a switch-over design can be utilized. For example, although the pilocarpine model showed an antiepileptogenic effect of celecoxib, a switch-over design revealed merely an antiseizure effect [7].

**Epileptogenesis beyond the latent period: a reevaluation of traditional concepts**

The classical understanding of epileptogenesis assumes that it occurs during the latent period, defined as the interval between an initial insult and the first unprovoked seizure. Contrary to this traditional view, contemporary research has established that epileptogenesis can continue well beyond the occurrence of the first unprovoked seizure [8]. The ‘kindling’ phenomenon, characterized by the establishment of increased seizure susceptibility through repetitive electrical or chemical stimuli, underscores the concept that repetitive seizures can enhance the probability of future seizures. This concept aligns with the “seizures beget seizures” (SBS) theory proposed by William Gower [9], further corroborated by the mechanism of secondary focus formation [10]. Experiments involving the ex vivo perfusion of kainic acid showed that infusion into one hemisphere could independently initiate epileptic activity in the contralateral hemisphere, providing empirical support for the SBS theory [11]. This evidence challenges the conventional confines of the latent period, indicating a more extended and dynamic process of epileptogenesis that necessitates a broader understanding of seizure development and propagation mechanisms.

**Systemic understanding of molecular and cellular alteration**

Epileptogenesis is triggered by an initial insult and perpetuated by repetitive seizures, as previously mentioned in this review. This process involves cellular damage, reactive astrogliosis leading to glial dysfunction, and microglial activation at the cellular level. On a molecular scale, factors such as the formation of excitatory synapses, imbalances in ions and water, compromised blood-brain barrier (BBB) integrity, synaptic dysfunction, and the presence of proinflammatory cytokines along with amyloid and phosphorylated tau proteins are also implicated in the pathogenesis of epileptogenesis. These elements interact in a complex and cooperative manner as depicted in Figure 1.

**Disinhibition**

Epileptogenic insults primarily lead to cellular damage; however, this damage is not a mandatory precursor for the development of epilepsy. For example, hyperthermia-induced epilepsy in animals does not necessarily result in cellular damage [12,13]. A key mechanism underlying epileptogenesis is the loss of inhibitory interneurons [14]. In the hippocampus, somatostatin-positive interneurons comprise over 50% of the hilar interneuron population. Their loss, coupled with an increase in synaptogenesis, has been linked to epilepsy in both animals and humans. Similarly, in the cortical area, parvalbumin-positive, fast-spiking basket cells play a crucial role in cortical inhibition. Supporting this is successful engraftment and seizure reduction following transplantation of human cortical interneurons in recent research [15], highlighting the critical importance of inhibitory actions in controlling seizures and epilepsy.
**Astrocytosis**

Nonspecifically, any form of damage or stimuli can trigger a reactive response in astrocytes, leading to an increase in their numbers. However, this proliferation is not the only concern. The astrocytes engage in complex communication with neighboring microglia, neurons, and blood vessels, contributing to the process of epileptogenesis [16]. The leakage of thrombin and albumin from the circulatory system activates astrocytes via the transforming growth factor beta (TGF-β) receptor. In addition, the production of proinflammatory cytokines and neurotrophic factors by microglia and neurons exacerbates the reactive state of astrocytes.

Astrocytes play a pivotal role in the dysfunction of the BBB and glymphatic system, mediated through the release of proinflammatory cytokines. This dysfunction is critical in the context of epilepsy because it can facilitate the abnormal neuronal excitability and connectivity that underlies seizure activity. Furthermore, astrocytes release gliotransmitters, such as glutamate, which can alter synaptic activity and further contribute to the neuronal imbalance between excitation and inhibition characteristic of epileptic circuits.

These interactions highlight the multifaceted role of astrocytes in epileptogenesis as passive responders to injury as well as active participants in the neuroinflammatory processes that promote the development and progression of epilepsy. Understanding these complex astrocytic responses and their interactions with other cell types within the brain is essential for developing targeted therapies that address the underlying mechanisms of epilepsy.

**Blood-brain barrier**

It is important to understand the composition of the BBB and its surrounding environment which includes capillary endo-

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**Figure 1** A schematic representation of cellular and molecular alterations associated with epileptogenesis

COX-2, cyclooxygenase; IL, interleukin; NMDAR, N-methyl-D-aspartate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GS, glutamine synthetase; PGE, prostaglandin E; TGF, transforming growth factor; AQP, aquaporin; MMP, matrix metalloproteinase; AMP, adenosine monophosphate; AK, adenosine kinase; EAAT, excitatory amino acid transporter; Kir, inwardly rectifying potassium channel.
theilial cells, astrocytes, and the perivascular space. Disruption of the BBB, as observed in acute brain injury or status epilepticus, facilitates the extravasation of serum albumin into the brain parenchyma. This albumin then binds to TGF-β receptors on astrocytes, leading to an increase in proinflammatory cytokines such as interleukin (IL)-1β and IL-6. In addition, the activity of an inwardly rectifying potassium channel (Kir4.1) is decreased. Because Kir4.1 channels in astrocytes redistribute extracellular potassium, their reduced activity can contribute to a hyperexcitable condition. A decrease in the excitatory amino acid transporters exacerbates this issue. Matrix metalloproteinases (MMPs) further aggravate BBB dysfunction and structural changes, increasing neuronal excitability and reducing the seizure threshold.

The role of the glymphatic system in this context is crucial and consists of astrocytes, perivascular space, and lymphatic drainage into the deep cervical lymph nodes. This system is recognized for its participation in clearing cerebral waste, especially in neurodegenerative diseases such as Alzheimer disease (AD). In epilepsy, quantitative magnetic resonance imaging (MRI) studies in patients with temporal lobe epilepsy (TLE) have shown an increase in enlarged perivascular space, indicating a connection between the glymphatic system and the pathophysiology of TLE [17]. Mechanistically, BBB disruption leads to neuroinflammation in the perivascular space and impairs glymphatic function, aggravating neuroinflammation by inhibiting the clearance of cytokines from the brain.

Nuclear factor kappa B (NF-κB) activation leads to an increase in toll-like receptor 4 (TLR4) and proinflammatory cytokines such as IL-1β, IL-6, and TNF-α. In addition, the activity of the Na+/K+/Cl− cotransporter in the choroid plexus is increased, leading to hypersecretion of cerebrospinal fluid (CSF). This affects the clearance mechanisms, drawing peripheral immune cells to the meningeal lymphatic system to activate microglia.

Astrogliosis results in the dysregulation of aquaporin-4 (AQP-4) on neuron bodies and synapses but not on vessels, leading to changes in polarity. The fundamental roles of AQP-4 include maintaining water homeostasis and contributing to potassium buffering in the brain, which are essential for normal neuronal function and prevention of excitotoxicity. Dysregulated AQP-4 expression can lead to imbalances in the extracellular environment, promoting neuronal hyperexcitability and increasing susceptibility to seizures.

Supporting the potential of targeting AQP-4 in epilepsy management, previous studies have demonstrated an increase in AQP-4 protein expression in patients with hippocampal sclerosis and TLE. Furthermore, acetazolamide, an AQP-4 inhibitor, has been shown to reduce the expression of multidrug resistance proteins. This effect indicates that acetazolamide and possibly other AQP-4 modulators could offer a novel therapeutic strategy for epilepsy, particularly in cases where drug resistance is a significant challenge.

### Neuroinflammation

Because the role of neuroinflammation in epileptogenesis has been addressed in numerous papers [18-22], a detailed description is not provided in the present manuscript. However, certain aspects of neuroinflammation in epileptogenesis are illustrated in the figures.

### Amyloid and tau

Dementia is one of the most common comorbid conditions in late-onset epilepsy (LOE), and the presence of dementia increases the risk of developing LOE [23]. Conversely, the presence of LOE increases the risk of developing AD, and the coexistence of LOE exacerbates cognitive dysfunction in patients with AD [24]. Evidence that approximately 40% of AD patients exhibit epileptiform discharges [25] supports the hypothesis of a shared pathogenic mechanism between these two conditions. In epilepsy, mouse studies have shown that tau knockout in an AD model suppresses amyloid β-induced epileptiform activity, revealing the crucial role of tau in epileptogenesis, alongside amyloid-β [26].

A recent study has found higher levels of tau and amyloid-β accumulation in the surgical specimens of drug-resistant epilepsy [27,28], indicating that tau and amyloid-β participate in the pathogenesis of epilepsy and are not merely byproducts of the degenerative process. This can be explained by glutamate toxicity between phosphorylated tau, amyloid-β, and seizures [29]. Amyloid-β increases glutamate secretion at synapses, leading to an increase in N-methyl-D-aspartate (NMDA)-mediated intracellular calcium, which induces an increase in extracellular amyloid. This increase in extracellular amyloid-β raises intracellular phosphorylated tau, further increasing amyloid-β levels. The heightened glutamate and activation of NMDA receptors lead to dysregulation of Ca2+-associated pathways, increasing p-tau and strengthening the cycle of increased excitability and neurodegeneration [30].
Mammalian target of rapamycin and brain-derived neurotrophic factor

Neurotransmitters, amino acids, growth factors, TLR ligands, and energy deprivation stimulate phosphoinositide 3-kinase (PI3K), which activates Akt. Activation of Akt leads to inhibition of tuberous sclerosis complex, inhibiting the mammalian target of rapamycin (mTOR) complex [31]. This cascade of events results in hyperactivation of the mTOR pathway, a phenomenon substantiated in both animal models and human epileptic tissues. Hyperactivation of the mTOR pathway is implicated in cellular proliferation, protein synthesis, immune responses, and neuronal excitability.

In the context of epileptogenesis, the mTOR inhibitor rapamycin was shown to prevent the development of spontaneous seizures in the kainic acid model of status epilepticus [32], as well as to reduce mossy fiber sprouting and cell loss. The mTOR pathway is intricately linked with the neuroinflammatory response [33]. Proinflammatory cytokines activate mTOR, which exacerbates astrogliosis, BBB breakdown, and mossy fiber sprouting. These alterations further induce the production of proinflammatory cytokines.

Brain-derived neurotrophic factor (BDNF) plays a pivotal role in neurogenesis, the expression of ion channels, neural differentiation, axonal remodeling, synaptic plasticity, neurotransmitter-related changes, and gamma-aminobutyric acid (GABA) receptor expression, all involved in epileptogenesis and ictogenesis. BDNF modulates the NF-κB through its receptor tropomyosin receptor kinase B (TrkB) and, subsequently, the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAP/ERK) pathway, augmenting neuroinflammation. Another proposed mechanism of epilepsy involves the BDNF-JAK/STAT pathway; BDNF induces the phosphorylation of JAK/STAT, diminishing GABAAergic action by modulating *Gabra1* gene expression.

The JAK2/STAT3 pathway is activated in the hippocampus as early as 1 hour following the induction of experimental status epilepticus [34], with activation of several known STAT3-regulated genes. Furthermore, blocking STAT3 phosphorylation during status epilepticus was shown to reduce the severity of subsequent seizures [35].

Temporal and spatial network formation

The traditional concept that identifies an “epileptogenic zone,” the resection of which could potentially cure epilepsy, is evolving with recognition of epilepsy as a network disorder. In this paradigm shift, excising the ‘primary’ epileptogenic zone and including the critical nodes within the network are essential for surgical interventions to be successful [36]. The formation of networks during epileptogenesis encompasses two critical dimensions: temporal and spatial synchrony [4]. Temporal synchrony involves the alignment in the timing of firing across adjacent neurons, which leads to seizures. This phenomenon was illustrated in a study utilizing organotypic hippocampal slice cultures in which the progression to epilepsy was effectively mapped through patterns of cellular activation [4]. Spatial synchrony pertains to the spatial arrangement of these synchronized networks. Electrophysiological studies have demonstrated that the activity of individual cells does not necessarily correlate with their spatial proximity. Initially, cells in close proximity exhibit a higher correlation in activity compared with those further apart. However, as epileptogenesis advances, a high correlation in activity is observed among both closely and distantly located cells, indicating the development of spatial network synchrony.

From a clinical standpoint, these aspects of synchrony reveal the intricate nature of epileptogenesis and emphasize the necessity of a holistic approach to epilepsy surgery. This approach should extend beyond the conventional focus on isolated zones to encompass a broader understanding and targeting of the network involved in seizure generation and propagation. Such a comprehensive strategy is necessary for enhancing surgical outcomes and providing more effective treatment avenues for individuals with epilepsy [37,38]. In a previous study, brain connections between the epileptogenic zone and propagation zone during the interictal period appeared to be strengthened [39], and the broader were the changes, the poorer the postsurgical results tended to be. More specifically, in a meta-analysis, a better outcome was observed in cases of extensive resection that included the temporal neocortex in TLE surgery compared with more restricted removal, such as selective amygdalohippocampectomy. This indirectly supports the existence of an epileptic network beyond the hippocampus [40].

Abnormal structural alterations, including synaptogenesis, reveal an imbalance between inhibitory and excitatory networks within the brain. Patch-clamp experiments have provided evidence supporting this hypothesis, demonstrating that, while cells may exhibit similar resting membrane properties, there is a disproportionate increase in excitatory synaptic currents compared with inhibitory currents [4]. This dis-
proportionality indicates that the synaptic changes contributing to the development of epilepsy are structural and involve significant functional shifts in neuronal activity, favoring excitation over inhibition.

**Epileptogenesis biomarkers**

Several blood biomarkers have been investigated in the context of epileptogenesis, encompassing a range of nonspecific cellular markers such as ubiquitin C-terminal hydrolase 1, neuronal-specific enolase, glial fibrillary acidic protein, and calcium-binding protein S100β, as well as neuroinflammatory markers MMP-9, high mobility group box 1 (HMGB-1), soluble intercellular adhesion molecule-5, and the CSF/serum ratio of IL-1β [41-43]. In addition, advancements in electroencephalography (EEG) biomarkers suggest indicators such as reduction in slow spindle duration, perilesional high-frequency oscillation, and epileptiform discharges as potential markers for posttraumatic epilepsy following traumatic brain injury [44]. Imaging studies have also been conducted to identify early indicators of epileptogenesis, with the T1ρ sequence of MRI notably predicting seizure susceptibility within the first 2 months after injury [45]. Microglia with translocator protein (TSPO) positron emission tomography imaging has been applied in both animal and human studies in which TSPO, primarily a marker of microglia, exhibited binding in the dentate gyrus 2 weeks post-status epilepticus induced by kainic acid, correlated with the frequency of subsequent spontaneous seizures [46].

MicroRNA (miRNA, miR) signatures in epileptic tissue exhibit diverse patterns contingent upon the experimental model. miRNAs, noncoding RNAs comprising approximately 20 nucleotides, play a pivotal role in posttranscriptional regulation of protein levels. Numerous studies have been performed to delineate miRNA patterns and identify pivotal miRNAs. A comprehensive synthesis of these findings is available in the preceding review literature. Notably, miRNAs such as 146a, 134, 132, 155, 203, and 124 were associated with epileptogenesis [47]. These miRNAs are implicated in the regulation of neuroinflammation, neurotrophic factors, ion channels, synaptic plasticity, and various transcription factors associated with epileptogenesis.

In another review of the literature, the common miRNAs among epilepsy, traumatic brain injury, and ischemic stroke, notably miR-21, miR-181a, and miR-155, were identified [48]. miR-21, implicated in cell development, apoptosis, and proliferation, lacks specificity for epilepsy and epileptogenesis. miR-181a, which influences immune response and protein modification, emerges as a potential marker for epileptogenesis. miR-155 participates in the neuroinflammatory process, cell proliferation, and apoptosis, and it is associated with the PI3K/Akt/mTOR signaling pathway. This pathway is well-documented in the context of epileptogenesis and ictogenesis. However, the clinical applicability of these findings is limited due to the variability of the data.

**Conclusion**

Beyond controlling seizures, the quest for drugs that can block the onset of epilepsy has been a goal for epileptologists, yet such a breakthrough remains elusive. However, achieving this objective is becoming more probable as insights broaden from just the structural changes caused by epileptogenic insult to a deeper molecular and genetic understanding. One example is a recent trial in which epilepsy was prevented with mTOR inhibitors in individuals with tuberous sclerosis who had not yet experienced seizures. Furthermore, harnessing tools such as functional MRI, stereo-EEG, and tractography is revolutionizing the understanding of epileptogenesis from a network perspective. These advancements can clarify the crucial networks involved and might provide the optimal time frame for applying treatments that could prevent epilepsy.

**Conflicts of Interest**

Kyung-II Park is the Editor-in-Chief of *Encephalitis*, and he was not involved in the review process of this article. No other potential conflict of interest relevant to this article was reported.

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