

Editorial: Cellular and Molecular Targets In Epileptogenesis Focusing on Disease Prevention

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Epilepsy is a complex disease characterized by the development of recurrent, unprovoked seizures, often associated with comorbidities, such as cognitive deficits, depression, anxiety and psychiatric disturbances, that worsen the patient's condition and mortality (Devinsky et al., 2018; Vezzani et al., 2019). Temporal lobe epilepsy (TLE) is the most common type of partial epilepsy in adulthood. Its most prevalent mesial form is characterized by seizures originating mostly in the hippocampus, but also in the amygdala and parahippocampal gyrus. Mesial-temporal lobe epilepsy (MTLE) is often accompanied by hippocampal sclerosis (MTLE-HS), commonly associated with antiseizure drug (ASD) resistance (Sloviter, 2005; Thom, 2014; Gambardella et al., 2016). The mechanisms underlying MTLE epileptogenesis are largely unknown, yet associated hereditary and environmental triggering factors, such as trauma, complex febrile seizures, *status epilepticus* (SE), inflammatory insults, or ischemia, are frequently detected. Disease onset and progression vary considerably with the putative triggering event and age. Several cellular mechanisms contribute to early epileptogenesis, such as altered synaptic plasticity and neuronal excitability, neuronal death, astrogliosis and astrocyte dysfunction, neuroinflammation, blood-brain barrier leakage, secondary non-convulsive SE and aberrant neurogenesis, yet their pathophysiological relevance is far from being understood. At least one-third of epileptic cases have no available medical treatment. This prompted the focus of research towards the understanding epilepsy etiopathogenesis and to the development of novel therapeutic strategies aiming at epileptogenesis prevention (Pitkänen et al., 2015; Cunha-Reis et al., 2021). This special issue highlights the discovery of new circulating biomarkers for early diagnosis and follow-up of epileptic cases and addresses new therapeutic strategies to mitigate early cellular events and the progression of epilepsy to avoid ASD resistance.

Pioneering studies showed that blood-brain barrier (BBB) breakdown generates a vicious cycle favouring epileptic seizures, while seizure-induced BBB disruption is critical to epilepsy onset and progression. Using the pilocarpine-induced SE model in the rat, the paper by [Mendes et al. \(2019\)](#), shows that altered BBB permeability to small molecules occurs within the first 4 hours of SE, preceding macromolecule leakage in the following 5 hours. While BBB leakage of macromolecules disappears within 24h, increased BBB permeability to small molecules persists, adding to brain damage over time. Therefore, the authors propose that there is a critical 24h temporal window of BBB dysfunction after the onset of the SE, during the acute phase, where therapeutic approaches should concentrate to prevent BBB damage and epileptogenesis.

Early and delayed BBB disruption leads to leakage of brain contents, which may provide biomarkers for the early diagnosis and progression monitoring of epileptogenesis. The paper by [Martins-Ferreira et al., \(2022\)](#) reviews the putative role of methylated circulating cell-free DNA

generated by neuronal apoptosis as a biomarker of ongoing epileptogenesis. Such an approach, relying on the cell-type specificity of DNA methylation (DNAm) to determine its tissue origin, is not currently used to approach epilepsy. Though it might be interesting, further studies are required to overcome the main limitations, such as the fact that early epileptogenesis depends on the triggering event and might not concur with neuronal apoptosis and cell death (Pitkänen et al., 2015). In line with this, [Berger et al. \(2022\)](#) review the role of DNAm changes as a trigger for early epileptogenesis alterations in gene expression (GE), extrapolating mainly on discoveries using the intracortical kainic acid rat model. The authors emphasize the role of glial cell DNAm in controlling early GE alterations involved in the regulation of neuronal death, reactive astrogliosis and brain inflammation and discuss how these processes could be targeted for the early prevention of epileptogenesis through directed epigenetic modifications.

[Baloun et al. \(2020\)](#) performed a cross-sectional study using hippocampal tissue from of MTLE-HS patients and from the Li²⁺-pilocarpine TLE animal model to uncover distinct miRNA profiles depending on the age of epilepsy onset in both patients and animal models. This analysis revealed overlapping miR-142-5p and miR-129-2-3p changes between MTLE-HS patients and rats with adult TLE onset. These miRNAs regulate immunomodulatory agents with convulsive and neuronal growth suppression properties that may be used in diagnosis. The study by [Leal et al. \(2022\)](#) extends this concept showing that low serum levels of circulating miRNA-22 correlate with overexpression of the proconvulsant ATP-sensitive ionotropic P2X7 receptor in the hippocampus and neocortex of MTLE-HS patients. These changes, which are more notorious in patients' refractory to three or more ASDs, seem to occur soon after the epileptogenic trigger and are not dependent on the age of onset and gender, making them useful as predictors of drug refractoriness. Interestingly, two P2X7 isoforms were identified in hippocampal and neocortical nerve terminals, being the higher MW (85KDa) isoform the most abundant in brain regions of MTLE-HS patients compared to the naturally occurring 67kDa receptor. This may denote post-translational protein modifications, which epileptogenesis implications are worth investigating in the future.

[Carvalho-Rosa et al., \(2023\)](#) used *in vitro* models of epileptiform activity (EA) to evaluate the time course of long-term potential (LTP) changes occurring within 30 min to 1h30m following EA while characterising the early modifications in synaptic structure leading to altered synaptic transmission patterns and neuronal excitability that may contribute to early epileptogenesis. The described synaptic molecular alterations in AMPA GluA1/GluA2 levels and AMPA GluA1 phosphorylation likely underlie the observed impaired post-seizure LTP and, notably, were associated modifications in synaptic lipid raft structure. Since lipid raft integrity is required for several molecular mechanisms involved in synaptic metaplasticity these may constitute promising targets for prevention of epileptogenesis.

[Zhang et al. \(2021a\)](#) show that altered expression of Par3, α PKC- λ , and Lgl1 proteins and enzymes participating in neuronal polarity definition and axonal growth during development are correlated with mossy fibre sprouting and neuronal cell loss in the CA3 region of the hippocampus from 3 days after kainic acid (KA)-induced SE in rats. While these findings suggest the involvement of Par3, α PKC- λ , and Lgl1 in early epileptogenesis their clinical translation requires further investigation. The group of [Zhang et al. \(2021b\)](#) reviewed preclinical studies and clinical evidence concerning the role of two glia-to-neuron signalling pathways, the high mobility group box-1 (HMGB1)/toll-like receptor 4 (TLR4) and interleukin-1beta (IL-1 β)/interleukin-1 receptor 1 (IL-1R1) pathways in the neuroinflammatory response contributing to brain injury in epilepsy. Although these signalling pathways were subject to investigation as therapeutic targets through the development of antibodies and inhibitors, the complete upstream and downstream links are still missing.

Finally, the papers by [Schulze et al. \(2020\)](#), [Tse et al. \(2021\)](#) and [Zhang et al. \(2022\)](#) go a step further by testing the impact of distinct therapeutic strategies in epileptic animal models. The first paper demonstrates the role of casein kinase 2 (CK2) activity in epileptogenesis in juvenile rats. Inhibition of CK2 activity before SE had a neuroprotective role on seizure onset, disease progression and chronic CA1 neuronal burst firing, by increasing $K_{Ca}2.2$ levels and function, also involving the upregulation of HCN1 and HCN3 channels. Tse et al. demonstrated that surgical implantation of depth electrodes increased brain and plasma molecules involved in epileptogenesis and neuroinflammation while it reduced the threshold for SE induced by KA. Implantation chronology aside, this suggests that electrode implantation in TLE patients may contribute to neuroinflammation, neurodegeneration and BBB leakage at the risk of accelerating disease progression. Zhang et al. report the neuroprotective role of subclinical anaesthetic doses of xenon gas against epileptogenesis in the pentylenetetrazole (PTZ) kindling model of TLE. These actions were evident early in the development of epileptogenesis and were probably due to the reduction of iron and oxidative and iron stress.

In summary, this special issue puts into evidence multiple targets for the early diagnosis as well as therapeutic interventions directed at epileptogenesis highlighting its benefits, caveats, and hurdles to clinical applications.

Keywords: blood-brain barrier, diagnosis, DNA methylation, epileptogenesis, inflammation, miRNAs, seizures, synaptic plasticity.

Abbreviations: ASDs, antiseizure drugs; BBB, blood-brain barrier; CK2, casein kinase 2; DNAm, DNA methylation; EA, epileptiform activity; GE, gene expression; HMGB1, high mobility group box-1; IL-1 β , interleukin-1beta; IL-1R1, interleukin-1 receptor 1; KA, kainic acid; MTLE, mesial temporal lobe epilepsy; SE, status epilepticus; TLR4, toll-like receptor 4.

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References:

- Cunha-Reis, D., Caulino-Rocha, A., and Correia-de-Sá, P. (2021). VIPergic neuroprotection in epileptogenesis: challenges and opportunities. *Pharmacol. Res.* 164, 105356. doi:10.1016/j.phrs.2020.105356.
- Devinsky, O., Vezzani, A., O'Brien, T. J., Jette, N., Scheffer, I. E., De Curtis, M., et al. (2018). Epilepsy. *Nat. Rev. Dis. Prim.* 4. doi:10.1038/nrdp.2018.24.
- Gambardella, A., Labate, A., Cifelli, P., Ruffolo, G., Mumoli, L., Aronica, E., et al. (2016). Pharmacological modulation in mesial temporal lobe epilepsy: Current status and future perspectives. *Pharmacol. Res.* 113, 421–425. doi:10.1016/j.phrs.2016.09.019.
- Pitkänen, A., Lukasiuk, K., Edward Dudek, F., and Staley, K. J. (2015). Epileptogenesis. *Cold Spring Harb. Perspect. Med.* 5. doi:10.1101/cshperspect.a022822.
- Sloviter, R. S. (2005). The neurobiology of temporal lobe epilepsy: Too much information, not enough knowledge. *C R Biol* 328, 143–153. doi:10.1016/j.crv.2004.10.010.
- Thom, M. (2014). Review: Hippocampal sclerosis in epilepsy: A neuropathology review. *Neuropathol. Appl. Neurobiol.* 40, 520–543. doi:10.1111/nan.12150.
- Vezzani, A., Balosso, S., and Ravizza, T. (2019). Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nat. Rev. Neurol.* 15, 459–472. doi:10.1038/s41582-019-0217-x.