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Molecular biomarkers in drug-resistant epilepsy: Facts & possibilities

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HIGHLIGHTS

- Molecular entities involved in epileptogenesis.
- Possibilities for accurately localizing epileptogenic zone prior to epilepsy surgery.
- Knife Technique for real-time identification of epileptogenic tissues.

Abstract

Despite great advances in our understanding of the process of epileptogenesis, we are yet to develop reliable biomarkers that have the potential to accurately localize the epileptogenic zone (EZ), to resolve the issue of heterogeneity in epilepsy surgery outcome. Inability to precisely localize the epileptogenic foci is one of the reasons why more than 30% of these DRE patients are not benefited. Molecular and cellular biomarkers in combination with imaging and electrical investigations will provide a more specific platform for defining epileptogenic zone. Potential molecular biomarkers of epileptogenesis including markers of inflammation, synaptic alterations, and neurodegeneration may also have the potential to localize EZ. At a molecular level, components derived from epileptic tissues, such as metabolites, proteins, mRNAs and miRNAs that are significantly altered can serve as biomarkers and can be clubbed with existing techniques to preoperatively localize the EZ. Neurosurgeons across the world face problems while defining the margins of the epileptogenic tissues to be resected during surgery. In this review, we discuss molecular biomarkers reported so far in the context of epileptogenesis and some unexplored markers which may have the potential to localize EZ during surgery. We also discuss the “Intelligent Knife” technique that couples neurosurgery and mass spectrometry allowing real-time characterization of human tissue and may prove to be instrumental in defining the margins of the epileptogenic zone during surgery.

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1. Introduction

Epilepsy is the most common serious neurological disorder and WHO has determined that epilepsy accounts for 1% of the global burden of disease, as measured by number of years lost due to disability or premature death [1]. It is estimated that ~30–40 percent of patients with epilepsy will not have complete seizure control with antiepileptic drug (AED) therapy alone [2]. Epilepsy cases that cannot be controlled with drug management are referred as drug-resistant epilepsy (DRE). Patients with localization related DREs undergo resective surgical therapy and have higher chance of attaining surgical freedom as compared with patients receiving medical treatment for DRE [3]. Pre-surgical evaluation of the epileptogenic zone (EZ) can be carried out using existing investigative techniques like EEG, VEEG, MRI, SPECT and MEG. Intraoperative coregistration of MRI, PET, electrocorticography (ECoG) and MEG provides better objective localization of the epileptogenic zone [3]. Despite using combination of all available invasive and non-invasive modalities the epileptogenic zone cannot be fully defined and in more than 30% of these cases the patients are not benefited, mostly due to inability to precisely localize the epileptogenic foci [3]. Molecular and cellular biomarkers in combination with imaging and electrical investigations may provide a more specific platform for defining epileptogenic zone (EZ). Thus, the challenge is to find a biomarker which can help accurately localize the epileptogenic zone complementing the imaging and electrical investigations.

Biomarkers of epilepsy will include factors that are very sensitive and specific and can be objectively determined and interpreted as indicators of pathological changes related to epileptogenesis and ictogenesis [4]. Molecular biomarkers indicating the presence, type, and severity of neuropathologically damaged tissue with epileptogenic potential may not only serve as biomarkers of epileptogenesis but may also have the potential for localizing EZ [5]. Epileptogenesis is the complex process of development of epilepsy and after a transient insult to the brain is accompanied by pathogenic processes including cell death, axonal sprouting, reorganization of neural networks, alterations in the release of neurotransmitters, and neurogenesis [6]. Various molecules associated with these pathological processes and specifically showing alterations either in their expression, localization and/or functions only in epileptogenic tissues may serve as potential biomarkers of localizing EZ. There are currently no validated molecular biomarkers that would allow the reliable localization of the epileptogenic zone. The well-defined resected brain tissue from focal epilepsy patients undergoing surgery are valuable and are ideal model systems for not only understanding the process of epileptogenesis but also development of novel biomarkers. It would be ideal to detect biomarkers that correlate with specific pathologic aspects of epilepsy as they may also give insights into defining epileptogenic zones preoperatively or during the surgery. Molecular biomarkers of epileptogenesis that are either up- or down-regulated, or altered, for example by phosphorylation and are specific for the epileptogenic focus can be imaged by selective PET tracers and used preoperatively to locate the EZ.

In this review we are discussing all possible molecular biomarkers reported so far in the context of epileptogenesis and some possible unexplored markers. We hypothesize that some of these biomarkers may have the potential to localize EZ during surgery. Although preliminary, but some ideas discussed here may be promising and may provide the basis for further studies. Cellular components derived from epileptogenic tissues, such as metabolites, proteins, miRNAs, mRNAs, and epigenetic alterations in DNA can serve as potential molecular biomarkers [7]. Genomics, proteomics, and metabolomics based approaches allow the surveillance of thousands of distinct molecular entities within a single biologic sample, thereby provide opportunities for development of biomarkers of epileptogenesis.

2. Differentially expressed genes and protein products as biomarkers

Several studies report differential regulation of gene expression and functional modulation of various proteins involved directly or indirectly in the process of synaptic alterations, inflammation and neurodegeneration. These molecules may not only serve as potential biomarkers of epileptogenesis but may serve as potential biomarkers for localizing EZ.

2.1. Molecules involved in abnormal synaptic transmission as biomarkers

During seizures the neuronal network goes awry leading to depolarization of neurons and excessive discharge of action potentials. The major cause this uncontrolled neuronal firing is the imbalance between excitatory and inhibitory synaptic transmission, which is a hallmark of epileptic seizures. Several immunohistochemical studies of brain specimens using antibodies against markers that label glutamatergic and GABAergic synaptic terminals indicates modulation of synaptic transmission in epileptogenic zone [8–10]. Even gene expression studies suggest changes in the mRNA levels of various glutamate and GABA receptor subunits [11].
there by modulating of glutamatergic and GABAergic synaptic transmission has [12]. Comparative studies of electrophysiological characteristics of neurons in maximally abnormal tissue with that in least normal tissues indicates significant alterations in synaptic current kinetics [13,14]. These changes in the cellular signaling properties may be associated with epileptogenesis. Investigating the mechanisms that regulate excitatory and inhibitory transmission at various levels is necessary to conceptualize epileptogenesis and to identify potential biomarkers for accurate localization of EZ.

There are considerable amount of evidences that indicate that modulation of inhibitory synaptic transmission mediated by GABA receptors leads to seizure generation. Dysfunction in GABAergic input causing reduced inhibition might contribute to epileptogenesis. GABAergic inhibition regulates the spread of epileptic discharges [15,16] and intrinsic burst firing properties of neurons [17]. In tissues resected from patients with MTLE loss of interneuron density has been shown to cause reduced GABAergic synaptic transmission [18]. There are reports where it has been shown that reduction in parvalbumin positive interneurons in tissues resected from patients with MTLE [19]. In case of focal cortical dysplasia (FCD) it has been reported that inhibitory transmission is reduced due to changes in the distribution of interneurons [20]. Moreover the duration of GABA-evoked currents is increased in FCD brain specimen indicating decreased release of GABA from the GABAergic terminals [21]. Quantitative changes in the subunits of GABA receptor [22], modulation of GABA by other neurotransmitters and second messengers [23,24], and phenotypic changes in GABA receptor types that create depolarizing rather than hyperpolarizing reactions to GABA [25] are also associated with epileptic deregulation. Thus, decrease in the GABA signalling allowing uncontrolled glutamate signalling cannot be solely implicated for epileptogenesis. An immature GABAergic inhibitory system has also been suggested to contribute to the process of epileptogenesis. A predominant GABAergic synaptic transmission in an immature neuronal network can cause depolarization leading to excessive cell firing, where GABA may be acting as an excitatory neurotransmitter [26,27]. It is represented by higher frequency of spontaneous inhibitory postsynaptic currents (IPSCs) and lower frequency of excitatory postsynaptic currents (EPSCs) as observed in dysmature cerebral development in severe cortical dysplasia [28]. Higher GABA inputs could also be contributed by cytomegalic interneurons [29] and by supernumerary cells in superficial layers and white matter observed in severe cortical dysplasia cases [30]. There is also possibility of increased GABA release and higher number of neurotransmitter release site in severe CD cases [31]. An increased GABA receptor activity relative glutamate receptor activity has been so far reported in severe CD cases and not in non-CD or mild-CD [12,32]. Histopathological features of tuberous sclerosis complex (TSC) cases are similar to severe CD cases, but the morphological and electrophysiological characteristics of neurons in resected brain samples from these two disorders vary significantly [33]. This indicates that molecular characteristics of TSC more closely resemble non-CD and mild-CD. These differences indicate that mechanism of epileptogenesis varies in patients with different epileptic syndromes. Thus GABA receptor activity may be specific for specific epilepsy pathology, thereby making it a potential biomarker (Table 1).

Excitatory neurotransmission mediated by glutamate leads to depolarization and excitation of target neurons through ionotropic receptors N-methyl-d-aspartic acid (NMDA) receptor, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor and kainic acid receptor. Post-mortem human brain studies have been performed to localize NMDA receptors, AMPA receptors and kainic acid receptors [33–35]. Tetramers of NMDA receptors is composed of GluN1, GluN2A–GluN2D and GluN3A–GluN3B [36] and has been extensively studied for its role in epilepsy. Mutations in the gene encoding GluN2A have been identified in patients with idiopathic epilepsy [37] and haplotypes of gene encoding GluN1 have also been associated with epilepsy [38]. Decreased GluN2B expression in pyramidal neurons has been shown in TLE patients and upregulation of this receptor subunit was reported in the pyramidal neurons in hippocampal sclerosis [9]. NMDA receptor mediated excitatory postsynaptic potentials recorded from dentate granule cells in brain specimens obtained from epileptic patients had increased duration and amplitude [39,40]. NMDA channel opening was enhanced even in the dissociated dentate granule cells from human epileptic hippocampus [41]. Moreover, NMDA-subtype glutamate receptor mediated excitatory postsynaptic current response is prolonged in slice preparations of surgically resected tissues obtained from temporal lobe epilepsy patients [42]. Thus, abnormal regulation of NMDA receptor-mediated glutamate receptor activity could be a major contributor for epileptogenesis. Further evidences indicate that NMDA receptors are involved in epileptogenesis and in epileptic tissues upregulation of NMDA receptor subunits has been shown to contribute to hyperexcitability [43]. One of our recent findings suggest that increase in the mRNAs of NMDA receptor subtypes [44,45] monorom from patients with MTLE (unpublished data). All these evidences indicate that NMDA receptors contribute to hyperexcitability and involved in the process of epileptogenesis. Thus glutamate receptor subtypes can serve as a possible biomarker for localization of EZ (Table 1).

In human brain nicotinic acetylcholine receptors (nAChRs) are also known to regulate excitatory and inhibitory synaptic transmission mediated by glutamate and GABA respectively. It has been reported that interneurons present in human cerebral cortex express α7 and α4β2 subtypes of nAChRs and that α4β2 nAChRs present on the pre-terminal regions of the interneurons contributes to GABA release process [21]. Inhibition of nicotinic cholinergic input to interneurons reduces GABAergic transmission to pyramidal neurons leading to increased excitability and seizures [21]. In mice nicotinic receptor desensitization inhibited nicotine-induced GABA release and seizures [44]. This suggests epileptic activity could be result of disinhibition of pyramidal neurons [21,44]. Another model describing the role of nicotinic receptor-mediated GABAergic inhibition is based on the fact that high doses of nicotine causes synchronous activation of interneurons [44]. Under physiological conditions interneurons generate synchronous oscillations, but during seizure there is entrainment of synchronous activity leading to activation of large populations of pyramidal neurons. It has been shown that oscillations during ictal events caused by widespread synchronous activity were blocked by GABAA receptor antagonist bicuculline [45,46], suggesting that epileptiform activity was induced by increased GABAergic transmission. Evidence exists that suggest the role of α7 and α4β2 nAChRs in nicotine-induced seizures [44]. Reduction in the nAChR function in the interneurons that synapse onto pyramidal neurons could contribute to the process of epileptogenesis. Nicotinic receptors enhance the release of glutamate in case of nicotine-induces seizures through activation of NMDA receptors [47]. Mutations in the genes encoding for α7 and α4β2 nAChR subtypes are known to be related to various forms of epilepsy [48–50]. Nicotinic receptors are known to provide excitatory input to the interneurons which in turn inhibit excitatory pyramidal neurons. Animal studies have shown that α7 antagonist methyllycaconitine (MLA) inhibited nicotine-induced seizures [44,45]. Moreover, α7 nAChR antagonists are known to block electroshock-induced seizures in mice and kindling-induces seizures in rats [51] further indicating that upregulation of α7 nAChR activity is involved in epileptogenesis. Under resting conditions in rat hippocampal slice...
Table 1

Summary of potential biomarkers for epileptogenesis.

<table>
<thead>
<tr>
<th>Category</th>
<th>Biomarker</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synaptic molecules</td>
<td>GABA receptors</td>
<td>An increased GABA receptor activity has been so far reported in severe CD cases and not in non-CD or mild-CD. GABA receptor activity may be specific for specific epilepsy pathology [12, 32].</td>
</tr>
<tr>
<td></td>
<td>Glutamate receptors</td>
<td>NRMDA receptors are involved in epileptogenesis and in epileptic tissues, upregulation of NMDA receptor subunits has been shown to contribute to hyperexcitability. Mutations in the gene encoding GluN2A have been identified in patients with idiopathic epilepsy and haplotypes of gene encoding GluN1 have also been associated with epilepsy. Decreased GluN2B expression in pyramidal neurons has been shown in TLE patients and upregulation of this receptor subunit was reported in the pyramidal neurons in hippocampal sclerosis [36, 37, 42, and 43].</td>
</tr>
<tr>
<td></td>
<td>Nicotinic acetylcholine receptors</td>
<td>Nicotinic receptors enhance the release of glutamate in case of nicotine-induced seizures through activation of NRMDA receptors. Mutations in the genes encoding for α7 and α4β2nAChR subtypes are known to be related to various forms of epilepsy. α7 nAChR antagonists prevent electroshock-induced seizures in mice and kindling-induce seizures in rats [48, 49, 50, 51].</td>
</tr>
<tr>
<td>Kinases</td>
<td>CDK5</td>
<td>Deregulation of CDK5 activity has been found in hippocampal sclerosis and in various forms of neuronal diseases. CDK5 levels were significantly higher in the anterior temporal neocortex of intractable epilepsy patients [54, 55].</td>
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<td></td>
<td>CSN2</td>
<td>In vivo treatment with the caspase kinase 2 inhibitor 4, 5, 6, 7-tetramethylrhodamine amplifies the slow after hyperpolarizing potential and prevents acute epileptiform activity [56].</td>
</tr>
<tr>
<td></td>
<td>TrkB</td>
<td>Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy in animal models [58].</td>
</tr>
<tr>
<td></td>
<td>ADK</td>
<td>ADK over expressed not only in a variety of rodent models of TLE, but also in epileptogenic tissues resected from human TLE patients [57].</td>
</tr>
<tr>
<td></td>
<td>m-TOR</td>
<td>m-TOR has been linked with many epilepsy syndromes, raising the possibility that second messengers in this signalling cascade may be useful biomarkers of epilepsy [75, 76].</td>
</tr>
<tr>
<td>Inflammatory molecules</td>
<td>C-reactive protein</td>
<td>CRP serum concentration was significantly higher in patients with refractory focal epilepsy than in controls. The most important predictor of increase in CRP level was secondarily generalized tonic-clonic seizure [60].</td>
</tr>
<tr>
<td></td>
<td>IL-1β</td>
<td>Increased mRNA levels of IL-1β have been observed in experimentally induced seizures. IL-1β levels rise in the acute phase during and shortly after SE; they fall during the latent period between a brain insult and chronic epilepsy onset, and then rise again once epilepsy develops [60, 64–66].</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>Elevated in serum and resected tissue within malformation of cortical development (MCD)/PCDs in TS patients [69, 70].</td>
</tr>
<tr>
<td></td>
<td>COX-2</td>
<td>Increased in neurons after traumatic brain injury and status epilepticus in animal models, and prevents CA1 pyramidal and interneuron excitotoxicity, suggesting a neuroprotective role [67, 68].</td>
</tr>
<tr>
<td></td>
<td>TGF-β/R</td>
<td>Blocking TGF-β/R in vivo reduces the likelihood of epileptogenesis in albumin-exposed rat brain, TGF-β/R acts as a possible therapeutic target and biomarker. Losartan prevents acquired epilepsy via TGF-β signalling suppression [77–79].</td>
</tr>
<tr>
<td>Epigenetic markers</td>
<td>miR-132</td>
<td>miR-132 was shown to be increased in the CA3 in an animal model of status epilepticus. In vivo microinjection of antagonams against miR-132-dependent miR-132 in CA3. A functional role of miR-132 in anti-inflammation has been identified. Since inflammation and blood–brain barrier opening are implicated in epileptogenesis, it is thought that increased miR-132 may contribute to epileptogenesis [87, 88].</td>
</tr>
<tr>
<td></td>
<td>miR-34a</td>
<td>Rapid upregulation of miR-34a after SE has been observed. Experiments with the miR-34a antagonist revealed that targeting miR-34a led to an inhibition of activated caspase-3 protein expression, which may contribute to increased neuronal survival and reduced neuronal death or apoptosis [90, 91].</td>
</tr>
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<td></td>
<td>miR-146a</td>
<td>Significantly increased expression in lesions compared with control tissue; negative feedback regulator of inflammatory processes in human glial cell culture. Increased levels during latent period follow SE in pilocarpine model [92, 93].</td>
</tr>
<tr>
<td></td>
<td>CREB, Mecp2, REST</td>
<td>Control transcription patterns of a set of genes involved in epigenetic modifications leading to altered neuronal excitability [95].</td>
</tr>
<tr>
<td></td>
<td>Reelin</td>
<td>Epilepsy-associated susceptibility for promoter methylation in a specific cohort of MTS patients that affects the reelin gene [99].</td>
</tr>
<tr>
<td>Metabolites</td>
<td>Myo-inositol</td>
<td>Increased levels in hippocampus during the latency phase in the pilocarpine and kainate models of epilepsy, and that this increase can be detected with 1H-magnetic resonance spectroscopy [103].</td>
</tr>
<tr>
<td></td>
<td>Glutathione</td>
<td>Decrease early after status epilepticus but subsequently increase gradually. Glutathione levels in the hippocampus, measured with 1H-MRS during epileptogenesis, correlate negatively with neuronal cell loss and with the frequency of recurrent seizures observed in the chronic epileptic stage [103, 104, 105].</td>
</tr>
<tr>
<td></td>
<td>N-acetyl aspartate</td>
<td>Level is decreased early following SE and in the latency phase, possibly reflecting neuronal loss and/or changed neuronal metabolism [103, 105].</td>
</tr>
<tr>
<td></td>
<td>Hemovanic acid</td>
<td>Significantly lower in CSF from patients with epilepsy versus controls [106, 107].</td>
</tr>
<tr>
<td></td>
<td>Hydroxyindolacetic acid</td>
<td>Significantly reduced levels of hydroxyindolacetic acid in CSF from patients with epilepsy versus controls [106, 107].</td>
</tr>
</tbody>
</table>

2.2. Protein kinases as biomarkers

Expression of cytoskeletal proteins, such as microtubule-associated proteins (MAPs), tubulin, and myoglobin, have been shown to be aberrant in intractable epilepsy. Kinases involved in phosphorylation of MAPs include glycogen synthase kinase-3β, Cyclin-dependent kinase 5 (CDK5), MAP kinase family members (ERK, P38) and caspase kinase 2 (alpha 1 polypeptide), among others. Of these kinases, MAP kinase, CDK5, and caspase kinase 2 (CK2) are abnormaly expressed in both DRE patients and animal models of epilepsy [54, 55]. Cyclin-dependent kinase 5 (CDK5) is involved in numerous neuronal functions and plays important role in maintaining homeostatic synaptic plasticity by regulating intracellular signalling cascades at synapses [54]. Deregulation of CDK5 activity is linked to various neurodegenerative diseases such as Alzheimer’s disease and also chronic loss of CDK5 is associated with seizures in animal model of epilepsy. Also genetic expression of CDK5 at animal model of epilepsy. Also genetic expression of CDK5 at different brain regions has been found to be increased in animal model of epilepsy [54]. Casein kinase 2 is thought to influence Wnt signalling via beta-catenin phosphorylation and the PI3-K signalling pathway via the phosphorylation of...
Akt. In one of our study CSNK2B the beta subunit of CK2 that serves regulatory functions was found to be downregulated (unpublished data). Previous reports have shown that inhibition of CK2 augments the slow after hyperpolarising potential suggesting CK2 inhibition indeed has anticonvulsive and perhaps antiepileptogenic properties [56]. Compelling evidence suggests that a focal dysfunction of adenosine signalling, caused by astrogliosis-induced over-expression of ADK, is sufficient to trigger focal electrographic seizures, possibly a very early event in the epileptogenic cascade that finally leads to the expression of spontaneous recurrent, clinical seizures. Likewise, deficient adenosine signalling was found in the hippocampus of kindled rats [57]. Studies provide proof of concept evidence that activation of TrkB kinase is required for the induction of chronic, recurrent seizures and anxiety-like behaviour after SE. This result provides a strong rationale for developing selective inhibitors of TrkB kinase for clinical use [58].

2.3. Molecules involved in inflammatory pathway

Brain inflammation plays a key role in the process of epileptogenesis [59]. Some of the candidate markers and pathways of brain inflammation are discussed here. Inflammatory proteins in the inflammasome blood have been proposed as potential markers of epileptogenesis in animal models. Plasma levels of inflammatory proteins like C-reactive protein, interleukin 1-beta (IL-1β), and interleukin 6 (IL6) were studied in the angular bundle stimulation model of the temporal lobe epilepsy [60]. Transcriptomic analysis of resected tissue from medically refractory focal epilepsy patients shows evidence of chronic inflammation in one study and other studies shows a more localized inflammatory response consisting of activated microglia, reactive astrocytes and endothelial cells [61]. Hallmarks of adaptive immunity such as T- and dendritic cell infiltrates are reported in focal cortical dysplasia [62]. Brain inflammation in Rasmussen's encephalitis demonstrates a peculiar involvement of cytotoxic T lymphocytes contributing to tissue pathology in concert with intrinsic brain cells [63]. Levels of IL-1β have been shown to rise in the acute phase during and shortly after SE; and fall during the latent period between a brain insult and chronic epilepsy onset, and then rise again once epilepsy develops. Also levels of miR-146a, a post-transcriptional inflammatory modulator, are shown to inversely associate with IL-1β during the acute seizure and latent phases [64]. IL-1β release from microglia and astrocytes within the epileptic focus, triggers more widespread inflammation and blood–brain barrier compromise shortly after seizures [65]. In a unilateral mesial temporal kainate injection mouse model of epilepsy, differential activation of IL-1β and IL-1 receptor antagonist was observed when comparing ipsilateral (side of later seizure onset) and contralateral mesial temporal lobes, suggesting this is a localized cytokine response specific to the site of epileptogenesis [66]. Cyclooxegenase (COX)-2 is another potential inflammatory mediator playing a dual role in neuroprotection and neurotoxicity, and in different time points during the process of epileptogenesis [67]. COX-2 expression is increased in neurons after brain insults such as TBI and SE in animal models, and prevents CA1 pyramidal and interneuron excitotoxicity, suggesting a neuroprotective role [58]. In a COX-2 conditional knockout mouse treated with pilocarpine, delayed mortality and cognitive performance (Morris water maze) were significantly improved after SE, compared with controls suggesting that COX-2 activation may lead to delayed neurodegeneration after SE, likely due to secondary inflammatory mechanisms [68]. In resected tissue IL-6 levels were shown to be elevated within malformation of cortical development (MCD)/FCDs in TS patients, suggesting this pathology may trigger a robust inflammatory response [69]. Another study shows high serum IL-6 levels in patients with intellectual disability and also who had a very high frequency of seizures [70]. A recent study reported significantly increased IL-6 levels with pilocarpine-induced SE in rats, but not after electrically kindled status [71]. The validity of IL6 as an epilepsy biomarker is still unclear.

2.3.1. TLR pathway

Toll-like receptor (TLR) expression is increased in FCD and tuberous sclerosis (TS) complexes. Two TLR agonists HMGB1 and IL-1β have been studied in these tissues. HMGB1 is released by neurons, activated astrocytes and microglia in response to physiological stress with subsequent binding to RAGE and TLR4 [72]. TLR4 activation further modulates NMDA receptor through aberrant subunit expression or post-translational phosphorylation leading to increased neuronal excitability [59,73].

2.3.2. mTOR pathway

Loss of function mutations in upstream regulators of mTOR are associated with dysplasias, epilepsy and neurodevelopmental disorders. Galanopoulou et al. have reviewed the association of mTOR pathway modulation with various forms of epilepsy in detail and suggested that various molecules of this signalling cascade may have the potential to serve as biomarkers of epilepsy. mTOR pathway is shown to be constitutively active in conditions with epileptogenic lesions, including TS and FCD. It has been suggested that both in TS and Type II cortical dysplasia the enlarged dysmorphic cells of varying neuronal or glial lineage known as balloon cells, demonstrate aberrant glutamate receptor expression patterns, likely resulting in hyperexcitability resulting in seizures [74]. Over-activation of the mTOR pathway have also been found in other etiologies of epilepsy, such as in traumatic brain injury, and in neonatal hypoxia-ischemia, non-genetic cases of infantile spasms and TLE are also reported however the association is not very clear [75]. mTOR inhibitors like rapamycin are being studied actively for their therapeutic potential in preventing seizures or epileptogenesis [76]. mTORC1 pathway includes various molecules like Vascular endothelial growth factor (VEGF), which is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. Ribosomal S6 kinase, part of the translational machinery believed to play a role in cell growth and proliferation; and EIF4E, a protein that binds mRNA and facilitates its delivery to ribosomes. These mTOR downstream targets may serve as epilepsy biomarker and need further investigations (Table 1).

2.4. Blood–brain barrier breakdown

BBB dysfunction is a hallmark of epilepticogenic brain injuries. BBB microvasculature damage during brain insults leads to extravasation of serum albumin into the cerebral cortex microenvironment, which activates a transforming growth factor-β receptor (TGFβR)–mediated signalling cascade in astrocytes causing local inflammation [70,86]. TGFβR2 a tumour suppressor gene shows upregulation in our lab (unpublished data) as well as previously in few other studies [77]. Canonical TGFβ signalling in astrocytes induces a pro-epileptic transcriptional activation of genes which leads to early dysfunction of astrocytes and delayed pathological hyper-excitability and seizures [78]. Losartan a FDA approved drug is shown to prevent acquired epilepsy via TGFβ–signalling suppression reflecting the significance of TGFβ–signalling in epileptogenesis [79]. Therefore TGFβR represents a potential biomarker of epileptogenesis (Table 1).

3. Epigenetic markers

Epigenetic alterations reported in epilepsy includes histone tail modifications [80–83], DNA methylation patterns [84,85],
microRNA (miRNA) expression [86–93] and transcription factor recruitment [88,89]. Several studies on miRNA profiling in epilepsy have identified the role of miRNAs which are key regulatory molecules in cells controlling protein levels in the pathogenesis of seizure-induced epilepsy both in animal models and human [86–93]. Levels of more than 100 different miRNAs were found to be altered in the hippocampus, of which more than 20 were identified in more than one study, including higher levels of miR-23a, miR-34a, miR-132 and miR-146a/miR-34and several other miRNAs like miR-21, miR-29a and miR-132, were identified as p53 regulated and also they get regulated after seizures formation [86]. miR-132 was shown to be increased in the CA3 in an animal model of status epilepticus. In vivo microinjection of antagonisms against miR-132 depleted miR-132 in CA3. A functional role of miR-132 in anti-inflammation has been identified [88]. Since inflammation and blood–brain barrier opening are implicated in epileptogenesis, it is thought that increased miR-132 may contribute to epileptogenesis [59]. Rapid upregulation of miR-34a after SE has been observed. Experiments with the miR-34a antagonist revealed that targeting miR-34a led to an inhibition of activated caspase-3 protein expression, which may contribute to increased neuronal survival and reduced neuronal death or apoptosis [89,90]. Significantly increased expression of miR-146a in lesions compared with control tissue; negative feedback regulator of inflammatory processes in human glial cell culture. Increased levels during latent period following SE in pilocarpine model [91,92]. Future studies in miR-132a, miR-34a and miR-146a are needed to investigate their potential as biomarker of Epilepsy. Recent studies have shown that transcription factors like REST (repressor element 1-silencing transcription factor), MeCP2 (methyl-CpG binding protein 2) and CREB (CRE binding element) may control the transcription patterns of a set of genes involved in epigenetic modifications leading to altered neuronal excitability and neuronal network that may eventually end up in the appearance of spontaneous seizures [94]. DNA methylation mediated changes in gene expression has been implicated in experimental and human temporal lobe epilepsies [95,96]. Decreased CREB levels suppress epilepsy. Altering CREB activity following a neurologic insult provides a therapeutic strategy for modifying epileptogenesis. Zhu et al reported increased Dnmt gene expression in the temporal neocortex of patients with TLE [97]. More recently, Miller-Delaney et al., described differential DNA methylation patterns in defining status epilepticus (SE) and epileptic tolerance [85]. Kobow et al. have shown increased promoter methylation in chronic TLE patients compared with autopsy controls. This group hypothesized that there is an epilyse-associated susceptibility for promoter methylation in a specific cohort of MTS patients that affects the Reelin gene. Within the cohort of TLE patients, there was a striking association between Reelin promoter methylation and the presence of GCD [99]. Therefore epigenetic regulation of gene expression in epilepsy is an exciting area for future basic as well as translational research and further studies are required to understand the mechanisms governing these epigenetic changes and finding specific epigenetic signatures of epileptogenesis.

4. Metabolites as biomarkers

Evaluation of brain metabolites associated with changes in brain metabolism during Epileptogenesis provides a complementary approach to the discovery of imaging biomarkers. PET measurement of glucose metabolism is already in clinical practice and several PET based studies in both the pilocarpine and kainic acid models of epilepsy shows hypometabolism in several brain areas during the latency phase and after the onset of recurrent seizures [100–102]. Also, the level of hypometabolism in the entorhinal cortex has been shown to be correlated with the development of recurrent seizures [103]. Metabolites other than glucose have been tested as potential imaging biomarkers. Levels of myoinositol, a metabolite linked to astrocyte activation, is shown to be elevated in the hippocampus during the latency phase in the pilocarpine and kainate models of epilepsy which can be detected with 1H-magnetic resonance spectroscopy. The level of myo-inositol reflects the extent of neuronal damage and neurodegeneration but it does not correlate with spontaneous recurrent seizures rat pilocarpine model [104]. Glutathione synthesized mainly in astrocytes is another metabolite and studies shows that levels of glutathione decrease early after status epilepticus (SE) but subsequently increase gradually [104,105]. Glutathione levels in the hippocampus, measured with 1H-MRS during Epileptogenesis were shown to correlate negatively with neuronal cell loss and with the frequency of recurrent seizures observed in the chronic epileptic stage [103,105]. Levels of lactate were shown to transiently increase during Epileptogenesis in animal models; however, there is no correlation with the frequency of seizures later on. Another brain cell-derived metabolite, N-acetyl-aspartate levels decreased early following SE and in the latency phase, possibly reflecting neuronal loss and/or changed neuronal metabolism [104,106]. Levels of several other metabolites like cyclic adenosine monophosphate, lactate, pyruvate, glycerol, glutamate, norepinephrine, homo-vanillic acid, hydroxyindolacetic acid, and N-acetylaspartate, as well as products of lipid peroxidation, such as F2-isoprostane, were shown to be altered after TBI and correlate with injury severity [106,107].

5. iKnife mass spectrometric lipidomics approach—an unexplored area in epilepsy surgery

Inability to precisely localize the epileptogenic foci is one of the reason why more than 30% of these DRE patients are not benefited. Problems are faced when defining the margins of the epileptogenic tissues to be resected during surgery. Balog and group have recently analysed the “smoke” created by electrosurgery (either monopolar or bipolar) by means of a new technique called rapid evaporative ionization mass spectrometry (REIMS) [108]. They have coined the term “intelligent knife” (iKnife) for this coupling of electrosurgery and mass spectrometry which allows near-real-time characterization of human tissue. In cases of tumour surgery when there is uncertainty about the resected margins, the removed tissue is sent to the pathology laboratory for intraoperative histological examination which is time consuming, costly and limited by few sampling points. REIMS technique circumvents these problems and has been shown to be successful in identifying statistically significant histologically specific mass spectral profiles of healthy and tumour tissues both in animal model as well as human in real time using [108].

The REIMS-based mass spectrometric method of tissue identification goes beyond identifying tumour versus healthy tissue by successfully identifying different tumour grades in several cases. REIMS technique may prove to be instrumental in defining the margins of the epileptogenic zone in real time during surgery. Initially surgically resected tissues with varying level of abnormalities defined by specific grading based on electrocorticography (ECoG) and imaging (MRI, SPECT/PET and MEG) data can be analysed ex vivo by REIMS for the construction of a histologically specific mass spectral database followed by analysis of smoke created by electrosurgery for differentiating the tissues with varying level of epileptogenicity (Fig. 1).

The real time identification of positive resection margins with specific mass specific profiles during surgery can improve the surgical outcomes by minimizing surgical trauma and the unnecessary
removal of healthy tissue. The iKnife mass spectrometric lipidomics approach not only is a potential alternative to histopathology in all cases where rapid diagnosis is preferred, but will also provide new chemical information on epileptogenic tissues with varying levels of abnormalities. As the technology becomes more widespread and the mass spectral database for epileptogenic tissues is created, it has the potential to reduce the cost of histopathology services and will also improve the surgical outcome.

6. Concluding remarks

Careful evaluation of the above-mentioned molecular entities on a larger sample size is needed to pinpoint their role in epileptogenesis and any distinguishing feature to be considered as a biomarker for localization of EZ. It is becoming increasingly clear, however, that identification of molecular biomarkers with clinical relevance will enhance our understanding of epilepsy in general and DRE in specific. Moreover it is possible that some of the biomarkers may also play a role in other neurological disorders, so a stringent control study is necessary. Elucidation of the molecules involved in epileptogenesis in the central nervous system and the periphery to define drug-resistance and accurately localize epileptogenic foci presents formidable methodological challenges but promises important new insights into epilepsy surgery.

Ethical approval

Not applicable.

Author contribution

Participated in study design: Aparna Banerjee Dixit, Manjari Tripathi, P. Sarat Chandra and Jyotirmoy Banerjee
Collected Data/Review of literature: AparnaBanerjee Dixit and Jyotirmoy Banerjee
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Conflict of interest

None.

Guarantor

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[98].

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