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Epigenetic mechanisms underlying human epileptic disorders and the process of epileptogenesis

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Abstract

The rapidly emerging science of epigenetics and epigenomic medicine promises to reveal novel insights into the susceptibility to and the onset and progression of epileptic disorders. Epigenetic regulatory mechanisms are now implicated in orchestrating aspects of neural development (e.g., cell fate specification and maturation), homeostasis and stress responses (e.g., immediate early gene transcription), and neural network function (e.g., excitation-inhibition coupling and activity-dependent plasticity). These same neurobiological processes are responsible for determining the heterogeneous features of complex epileptic disease states. Thus, we highlight recent evidence that is beginning to elucidate the specific roles played by epigenetic mechanisms, including DNA methylation, histone code modifications and chromatin remodeling, non-coding RNAs and RNA editing, in human epilepsy syndromes and in the process of epileptogenesis. The highly integrated layers of the epigenome are responsible for the cell type specific and exquisitely environmentally responsive deployment of genes and functional gene networks that underlie the molecular pathophysiology of epilepsy and its associated co-morbidities, including but not limited to neurotransmitter receptors (e.g., GluR2, GLRA2, and GLRA3), growth factors (e.g., BDNF), extracellular matrix proteins (e.g., RELN) and diverse transcriptional regulators (e.g., CREB, c-fos, and c-jun). These important observations suggest that future epigenetic studies are necessary to better understand, classify, prevent and treat epileptic disorders.

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Keywords

chromatin; DNA methylation; epigenetic; epilepsy; histone; long non-coding RNA (lncRNA); microRNA (miRNA); non-coding RNA (ncRNA); repressor element-1 silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF); RNA editing

INTRODUCTION

Intensive research efforts seeking to characterize the natural history of a variety of epileptic disorders and their responses to different treatment modalities have revealed that epilepsy is a very complex and heterogeneous continuum of disease states (Jacobs et al., 2009). The susceptibility to and onset and progression of epilepsy is influenced by a range of inherited and acquired factors, including those that are known as well as others that have yet to be recognized. These elements likely play diverse roles in the determination of central nervous system (CNS) structure, function, and plasticity that are ultimately responsible for producing a wide spectrum of epileptic signs, symptoms and associated co-morbidities (Andrade, 2009; Jacobs et al., 2009; Rakhade and Jensen, 2009). Over the past few decades, genetic studies in humans and in animal models have led to the identification of causative genes for various epileptic syndromes. These genes are largely implicated in the mediation of brain development and neuronal excitability (Andrade, 2009). These genes are associated with syndromes that are characterized principally by brain malformations (e.g., schizencephaly, lissencephaly, subcortical band heterotopia, periventricular nodular heterotopia, polymicrogyria, and focal cortical dysplasia) and those that are not (e.g., juvenile myoclonic epilepsy, childhood absence epilepsy, genetic epilepsy with febrile seizures plus, severe myoclonic epilepsy of infancy and severe myoclonic epilepsy borderland, and temporal lobe epilepsy) (Andrade, 2009).

Additional studies have assessed risk factors that predict the development of epilepsy because of acquired diseases including trauma, CNS infections, hypoxic-ischemic and metabolic disorders, tumors, and vascular abnormalities (Jacobs et al., 2009). Complementary studies have also begun to elucidate the molecular and cellular basis for epileptogenesis, the process that leads to the onset and progression of unprovoked seizures in a previously healthy brain (Jacobs et al., 2009; Rakhade and Jensen, 2009). Epileptogenesis is associated with complex temporal and spatial abnormalities of neural network structure and activity mediated by post-translational modifications of proteins, activation of immediate early genes (IEGs), and other alterations in profiles of gene expression and function (e.g., GABA_A receptor subunit, CREB, JAK-STAT, BDNF, and EGR3) that eventually lead to deregulated neural circuits with a predisposition for synchronous electrical activity (Rakhade and Jensen, 2009). Because of this evolution in our understanding of epilepsy, the nosology of epileptic disorders is continually being revised with the aim of encompassing not only clinical and phenomenological descriptions but also more modern genetic and mechanistic etiological classifications (Capovilla et al., 2009). In addition, these discoveries are also promoting the development of new therapeutic strategies based on genetic and other pathogenic factors. Despite these important advances, however, many questions regarding the neurobiology of epilepsy and its optimal prevention and treatment remain unanswered (Jacobs et al., 2009). In particular, defining and selectively modifying the complex relationships between causative genetic and environmental factors and the final common pathways for epileptogenesis that precipitate distinct subtypes of epilepsies remain critical challenges for future enquiries.

Recent studies have established that highly interconnected epigenetic regulatory networks play essential roles in modulating every aspect of cellular and organismal development, homeostasis, and aging and even participate in transgenerational inheritance (Mehler, 2008). By serving as the exquisitely environmentally sensitive molecular machinery that orchestrates the specific deployment of genes and gene networks within every cell in the body, epigenetic

mechanisms act as the interfaces for mediating gene-environmental interactions and as the higher order control systems for properly executing sophisticated regulatory programs embedded within the genome. Thus, epigenetic mechanisms are responsible for the phenotypes acquired during developmental critical periods and for complementary changes that occur throughout life. In the CNS, epigenetic regulation is important for mediating the interplay between cell intrinsic processes and complex spatiotemporal patterns of local and long distance environmental cues that dynamically sculpt brain development and the structure and function of neural networks throughout life (Mehler, 2008). Indeed, epigenetic mechanisms are critical for promoting brain patterning, neural stem cell (NSC) maintenance and proliferation, neurogenesis and gliogenesis, cellular migration, and synaptic and neural network connectivity and plasticity and are also implicated in orchestrating sophisticated cognitive functions including learning and memory (Mehler, 2008).

The major epigenetic mechanisms include DNA methylation, histone code modifications and chromatin remodeling, the deployment and actions of non-coding RNAs (ncRNAs) and RNA editing (see below). Not surprisingly, these epigenetic processes are particularly susceptible to disruption during gestation, the neonatal period, puberty and senescence and are involved in the pathophysiology of many complex diseases that manifest during these critical periods (Mehler, 2008). Epilepsy is no exception (Mehler, 2008; Urdinguio et al., 2009). Though, to our knowledge, the importance of epigenetic mechanisms in epilepsy has not previously been discussed systematically or in detail. In this review, we highlight evidence that suggests epilepsy is characterized not only by alterations in genetic and environmental factors but also by a spectrum of dysfunctional epigenetic factors and processes. These observations imply that research efforts in the post-genomic era should focus on better characterizing the normal and aberrant roles of epigenetic factors in the pathogenesis of epileptic disorders and of potentially modifiable epigenetic gene regulatory mechanisms during the process of epileptogenesis. We hope that these investigations will lead to the discovery of new biomarkers for risk stratification and molecular diagnosis and to the development of novel treatment paradigms for preventing and even reversing epilepsy.

EPIGENETIC MECHANISMS IN EPILEPTIC DISORDERS AND THE PROCESS OF EPILEPTOGENESIS

DNA methylation

DNA methylation has a range of cellular functions that include regulating global and local gene transcription as well as maintaining genomic integrity, facilitating X chromosome inactivation and mediating associated parent-of-origin effects (Robertson, 2005). Differential DNA methylation of gene promoter regions is accountable, in part, for the dynamic modulation of cell-, tissue-, and developmental stage-specific gene expression profiles that promote cell identity and function throughout life. Consequently, this epigenetic mechanism is important for all aspects of CNS development, homeostasis, plasticity, and injury responses; and aberrant DNA methylation profiles are associated with a wide array of disease states including neurodevelopmental, neuropsychiatric and neurodegenerative disorders as well as cancer, which are all co-morbidities in numerous epileptic syndromes (Mehler, 2008; Urdinguio et al., 2009).

DNA methylation is catalyzed by DNA methyltransferase (DNMT) enzymes that transfer methyl groups from S-adenosylmethionine (SAM) to cytosine residues resulting in the formation of 5-methylcytosine, primarily at CpG dinucleotide-containing regulatory sequences (Robertson, 2005). These DNA methylation events result directly in the inhibition of gene transcription and indirectly in transcriptional silencing that is mediated by methyl-CpG-binding domain proteins (MBDs). Members of the DNA methyltransferase enzyme

family include DNMT3a and DNMT3b, which promote *de novo* methylation, and DNMT1, which maintains methylation marks. Methyl-CpG-binding domain proteins, such as MeCP2, are DNA binding proteins that participate in gene regulation at methylated genomic loci by recruiting additional epigenetic regulatory factors, which modulate local and long-range chromatin structural and functional dynamics (see below). The expression and function of DNA methyltransferases and methyl-CpG-binding domain proteins are exquisitely regulated throughout neural cell fate specification, maturation and survival and during activity-dependent synaptic plasticity, which are all processes that can be associated with the pathophysiology of epilepsy (Sharma et al., 2008).

Indeed, recent evidence highlights the key roles played by DNA methylation factors in the pathogenesis of epileptic disorders. For example, Rett syndrome (RS) is a progressive X-linked autism spectrum disorder (ASD) typically associated with infantile spasms (IS) or other severe epileptic syndromes. Rett syndrome is primarily caused by mutations and duplications in MeCP2 that result in aberrant expression and function of this methyl-CpG-binding domain protein (Amir et al., 1999). Intriguingly, MeCP2 has a number of context-specific global and more selective effects on gene expression in the CNS. MeCP2 mediates global transcriptional repression by associating with the Sin3a, NCoR and c-Ski transcriptional co-repressor complexes; global transcriptional activation through interactions with the transcriptional activator, CREB; and selective regulation of activity-dependent BDNF transcription. MeCP2 also has additional molecular functions that link DNA methylation with other epigenetic processes such as higher order chromatin organization (see below) and post-transcriptional RNA processing (e.g., RNA modifications, quality control and transport). For example, MeCP2 promotes the formation of chromatin loops within the imprinted Dlx5–Dlx6 genomic locus and mRNA splicing by interacting with YB1, a multifunctional regulatory factor with roles in modulating gene transcription and post-transcriptional RNA processing (Young et al., 2005). These observations imply that epileptic syndromes associated with MeCP2 gene disruption are mediated by complex genome-wide as well as local epigenetic dysregulation. In fact, through direct and indirect mechanisms, MeCP2 regulates genes implicated in neurobiological processes that are relevant for the pathogenesis of epileptic disorders and related co-morbidities (Urduingio et al., 2008). For example, an examination of brain tissues from a Rett syndrome animal model revealed deregulation of factors responsible for neurotransmitter biosynthesis and for promoting the differentiation and maturation of various neural cell types (Urduingio et al., 2008).

Emerging evidence is also starting to elucidate the roles played by DNA methylation in the pathophysiology of other epileptic syndromes including temporal lobe epilepsy (TLE) and Prader-Willi and Angelman syndromes (PWS and AS). High levels of DNA methylation in the promoter region of the critical neural factor, Reelin (RELN), are associated with the pathogenesis of temporal lobe epilepsy (Kobow et al., 2009). RELN is an extracellular matrix molecule that plays an important role in cell positioning and neuronal migration during brain development and in synaptic function and plasticity during adult life. In the hippocampus, RELN is essential for the proper migration and laminar patterning of granule cells during development and for the maintenance of the integrity of the dentate gyrus (DG) during adulthood. Moreover, RELN protein levels are decreased in hippocampal regions of human temporal lobe epilepsy specimens and are responsible for causing granule cell dispersion (GCD), a pathological feature of the DG that is often found in temporal lobe epilepsy (Haas and Frotscher, 2009). A number of studies have shown that RELN expression is subject to regulation by DNA methylation (Levenson et al., 2008). Thus, Kobow et al. examined RELN gene promoter methylation in hippocampal subregions (i.e. molecular and granule cell layers of the DG and presubiculum) from human temporal lobe epilepsy specimens. RELN promoter methylation was found to be greater in temporal lobe epilepsy specimens than in controls and displayed significant correlation with GCD, implying that aberrant RELN promoter

methylation plays a significant role in the pathogenesis of temporal lobe epilepsy (Kobow et al., 2009).

DNA methylation is also important for genomic imprinting, and functional and cytogenetic abnormalities in paternal or maternal 15q11–13 alleles lead, respectively, to Prader-Willi and Angelman syndromes, which are autism spectrum disorders with epileptic phenotypes. Genes within this genomic region include UBE3 and GABA receptor subunits (e.g., GABRB3) that are implicated in epileptogenesis. Both of these syndromes exhibit abnormalities of DNA methylation and expression of genes within this cluster (Hogart et al., 2009; Hogart et al., 2007). Furthermore, MeCP2 binds to methylated gene promoters within this region and also to the methylated RELN promoter suggesting that MeCP2 is important for selective regulation of these genes and highlighting the complex role of epigenetic mechanisms in mediating diverse epileptic disorders, such as Rett syndrome, temporal lobe epilepsy, and Prader-Willi and Angelman syndromes.

Dynamic alterations in DNA methylation patterns are also found during the induction of epileptogenesis in animal models. For example, the deregulation of BDNF expression is implicated in the mechanisms underlying epileptogenesis, and the expression of BDNF is regulated by various cellular processes including methylation of the BDNF promoter (Aid et al., 2007). In addition, the inhibition of DNA methyltransferases in hippocampal neurons results in the suppression of neuronal excitability and network activity (Nelson et al., 2008). These effects are modulated in part by NMDA receptor-mediated activity-dependent demethylation of the BDNF promoter. Intriguingly, these effects of DNA methyltransferase inhibition on spontaneous excitatory neurotransmission require the presence of MeCP2, once again suggesting the singular importance of this methyl-CpG-binding domain protein in synaptic and neural network processes important for the epileptic state (Nelson et al., 2008).

Chromatin architecture and dynamics

Chromatin is not simply responsible for packaging DNA within the cell nucleus. It is also critical for actively controlling DNA accessibility; local and long range interactions between genes, gene clusters and regulatory elements; and the compartmentalization of genomic and epigenetic factors and processes within the nucleus (Cairns, 2009; Jenuwein and Allis, 2001; Kouzarides, 2007). Modulating chromatin structure and function at single nucleotides, specific gene loci, and more extensive genomic regions, therefore, represents an important epigenetic mechanism for promoting the execution of a broad array of genomic programs including DNA replication, DNA repair and recombination, maintenance of genomic integrity, inactivation of discrete regions of chromosomes and entire chromosomes, and regulation of gene expression. Like evolving profiles of DNA methylation, dynamic modulation of chromatin in neural cells is similarly implicated in mediating almost every aspect of nervous system development, homeostasis and plasticity, and abnormalities in chromatin structure and function are also associated with a range of neurodevelopmental, neuropsychiatric and neurodegenerative disorders as well as cancer (Mehler, 2008; Urduingio et al., 2009).

The basic unit of chromatin is the nucleosome, which consists of a DNA molecule that is wrapped around an octamer of core (e.g., H2A, H2B, H3, H4), linker (e.g., H1) and variant (e.g., H2A.X, H3.3) histone proteins. This nucleosome structure forms the basis for local and more global chromatin architecture and dynamics, which are subject to modulation by a series of integrated epigenetic mechanisms that include histone modifications, nucleosome repositioning, and higher order chromatin remodeling. Histone modifying enzymes catalyze post-translational changes to histone protein N-terminal domains including but not limited to acetylation, methylation, phosphorylation, biotinylation, ubiquitylation, SUMOylation, and ADP-ribosylation; and each of these affects DNA-histone interactions and may have a specific functional consequences (Jenuwein and Allis, 2001). For example, certain modifications of

histones in gene promoter regions have the potential to activate or repress gene transcription. The “histone code” refers to the cumulative profile of histone modifications that cooperatively establish functional microdomains in the nucleus (Jenuwein and Allis, 2001). Nucleosome repositioning enzymes also modify the conformation of DNA and histone proteins promoting the movement and reorganization of gene regulatory regions and more widespread chromatin remodeling (Cairns, 2009). Chromatin also forms higher order structures that include more loosely packaged euchromatin, which is relatively open and transcriptionally active, and more compact heterochromatin, which is transcriptionally inactive but has diverse structural roles including regulation of numerous developmental processes and the maintenance of genomic integrity (Kouzarides, 2007).

These important epigenetic mechanisms are implicated in the molecular pathophysiology of various epileptic disorders. In fact, a spectrum of clinical syndromes that manifest with epilepsy is caused by abnormalities in factors explicitly responsible for chromatin regulation. For example, biotin deficiency is characterized by epilepsy as well as other clinical features: hypotonia, ataxia, mental retardation and fetal malformations (Zemleni et al., 2008). Biotin serves as an essential coenzyme in the metabolism of fatty acids, amino acids and glucose and recent studies suggest that it also plays a role in chromatin regulation through histone biotinylation (Hassan and Zemleni, 2006). In addition, ATRX is a member of the SWI/SNF family of chromatin remodeling enzymes that interacts with a number of other epigenetic factors, including MeCP2, and is involved in diverse functions including transcriptional regulation, heterochromatin formation, DNA repair and chromosome segregation (Gibbons et al., 2000). Mutations of the ATRX gene lead to alpha thalassemia mental retardation (ATR-X), an X-linked mental retardation (XLMR) syndrome associated with epilepsy in approximately 30% of patients (Gibbons, 2006). These patients exhibit abnormalities in profiles of DNA methylation and chromatin structure. The epileptogenic mechanisms may include perturbations of inhibitory interneuron survival and differentiation, which are partly mediated by ATRX activity, and, therefore, to the deployment of neural networks with an altered balance between excitatory and inhibitory components (Medina et al., 2009). Moreover, NSD1 is a histone methyltransferase that is involved in chromatin regulation. Mutations and deletions of the NSD1 gene are responsible for various overgrowth phenotypes including some cases of Beckwith-Wiedemann and Weaver syndromes as well as most cases of Sotos syndrome (Turkmen et al., 2003). The cardinal features of Sotos syndrome include macrocephaly, an increased risk of tumors and neurological abnormalities, particularly epilepsy (Baujat and Cormier-Daire, 2007).

Furthermore, KDM5C (JARID1C/SMCX) is a histone demethylase enzyme that plays a role in chromatin remodeling and gene regulation. Mutations in KDM5C are associated with epilepsy as well as X-linked mental retardation, short stature and hyperreflexia (Abidi et al., 2008; Tzschach et al., 2006). These effects are mediated by interactions between KDM5C and the master epigenetic regulator-Repressor Element-1 Silencing Transcription Factor / Neuronal Restrictive Silencer Factor (REST/NSRF) (Tahiliani et al., 2007), which is responsible for orchestrating context-specific CNS transcriptional regulation by dynamic recruitment of macromolecular complexes of epigenetic factors to diverse genomic sites (Qureshi and Mehler, 2009). In fact, REST is a molecular platform to which a diverse array of factors may be recruited for participation in epigenetic remodeling of DNA, histones, nucleosomes, and higher-order chromatin codes. Intriguingly, REST is responsible for regulating the expression of various factors implicated in epileptogenesis, including growth factors, ion channels, neurotransmitter receptors, gap junctions and neurosecretory vesicles, as well as those involved in seminal neural developmental processes and adult neurogenesis (Qureshi and Mehler, 2009). In this regard, our recent studies have uncovered neuronal and glial subtype- and developmental stage-specific profiles for REST target genes including many that are associated with human epilepsy syndromes (e.g., Apoe, Arx, Brd2, Cacnb4, Cacng3, Chrn2, Clcn2, Crh, Gabrd, Gabrg2, Prnp,

and Tsc1) (Abrajano et al., 2009a; Abrajano et al., 2009b). PRICKLE1/RILP (REST interacting LIM domain protein) is a nuclear envelope protein that is involved in the nuclear targeting of REST and in the non-canonical Wnt/beta-catenin developmental signaling pathway. A PRICKLE1 mutation that blocks PRICKLE1/REST interactions causes progressive myoclonus epilepsy (PME), a syndrome characterized by myoclonic seizures, generalized seizures, ataxia and dementia (Bassuk et al., 2008). PRICKLE1 is expressed in brain regions involved in epilepsy and ataxia in mice and humans and may also play a role in modulating neuritogenesis (Fujimura et al., 2009; Okuda et al., 2007).

These examples illustrate important relationships between chromatin regulatory factors and human epileptic syndromes. Indirect evidence also demonstrates that the dynamic expression and function of chromatin regulatory factors are relevant for the molecular pathophysiology of epilepsy. For example, a mouse model engineered without the deacetylase domain of histone deacetylase 4 (HDAC4) exhibits seizures as mice mature beyond 5 months of age, with seizures elicited by handling suggesting an important pathogenic environmental trigger (Rajan et al., 2009). Moreover, PIM3 is an activity-dependent kinase with histone phosphorylase activity that is induced in specific regions of the mouse hippocampus and cortex in response to kainic acid and electroconvulsive shock-induced seizures (Feldman et al., 1998). Levels REST and its isoform, REST4, increase in response to seizures in animal models of epilepsy and regulate factors, such as neuropeptide substance P (NPY) and neurokinin B (NKB), that may play operative roles in epilepsy (Gillies et al., 2009).

The functional consequences of changes in chromatin regulatory factor expression and function are also highlighted by the spectrum of chromatin alterations being found in animal models of epileptogenesis, such as drug administration- and electroconvulsive seizure-induced epilepsies. These profiles include both gene selective and more global alterations in chromatin remodeling and correlate with changes in gene expression. For example, three epileptogenic agents—SKF82958 (a dopaminergic receptor agonist), pilocarpine (a muscarinic acetylcholine receptor agonist) and kainic acid (a kainic acid glutamate receptor agonist)—induce chromatin remodeling in hippocampal neurons, including transient phosphorylation of histone H3 at serine 10 (H3S10) and acetylation at lysine 14 (H3K14) (Crosio et al., 2003). These histone modifications are coupled with the engagement of mitogen activated protein kinase (MAPK) pathways and immediate early gene transcription (e.g., c-fos), which are mechanisms that mediate epileptogenesis. Additionally, in primary cultured cortical neurons, the glutamate receptor, GluR2, is epigenetically regulated after kainic acid exposure by rapid induction of promoter region histone H3 and H4 deacetylation resulting in mRNA downregulation mediated by REST (Jia et al., 2006).

Further, kainic acid induces a rapid but transient phosphorylation of histone H3 in neurons of the dentate gyrus and a more widespread and sustained histone H4 acetylation in neurons of the hippocampus (Sng et al., 2006; Taniura et al., 2006). Kainic acid also stimulates the expression of the histone acetyltransferase, CREB-binding protein (CBP), which correlates with histone H4 hyperacetylation in the hippocampus. Moreover, kainic acid also induces histone acetylation and phosphorylation in the c-fos gene promoter and histone acetylation in the c-jun gene promoter. Further, the spatiotemporal profiles c-fos and c-jun gene expression in the hippocampus after kainic acid administration correlate with histone H4 hyperacetylation, and these histone modifications are associated with the c-fos gene promoter (Sng et al., 2006; Taniura et al., 2006). These observations imply that the expression of these immediate early genes is regulated by gene-specific histone modifications. This conclusion is supported by the finding that treatment with Trichostatin A (TSA), a potent histone deacetylase (HDAC) inhibitor, leads to histone hyperacetylation and an increase in the expression of these genes after kainic acid administration (Sng et al., 2005). Interestingly, in these studies, pretreatment with curcumin, which also inhibits specific histone modifying enzymes, attenuates immediate

early gene expression and the severity of status epilepticus after kainic acid treatment (Sng et al., 2006; Taniura et al., 2006).

Other studies in electroconvulsive-seizure-induced models of epilepsy demonstrate that the CREB gene promoter is subject to selective histone H4 and H3 modifications (Tsankova et al., 2004). CREB is an important transcriptional activator that is implicated in modulating a broad array of cellular processes including the differential expression of GABA_A receptor subunits in the hippocampus, which is important for epileptogenesis. This observation suggests that epigenetic modulation of transcription of key neurotransmitter receptors involved in orchestrating the interplay between synaptic excitation and inhibition is involved in mediating epileptogenesis. Moreover, the induction of seizures is associated with BDNF gene promoter histone H4 hyperacetylation and increased expression of BDNF (Tsankova et al., 2004). Further, histone deacetylation contributes to the differential expression of alternative BDNF transcripts (Aid et al., 2007).

Regulatory non-coding RNAs (ncRNAs)

Eukaryotic genomes clearly give rise to non-protein-coding RNAs (ncRNAs) that transact a vast array of cellular functions (Amaral et al., 2008; Mattick et al., 2009). The versatility of these ncRNAs for orchestrating and catalyzing numerous diverse biological processes depends on biophysical and biochemical properties including RNA structure, folding and bioenergetics, as well as intracellular and intercellular mechanisms that promote the specialized functions and the localization of RNA molecules. Because of these flexible and robust features, ncRNA molecules are capable of very sophisticated coordination of cellular functions including dynamic coupling of nuclear and cytoplasmic processes in a developmentally regulated, environmentally responsive manner. These ncRNAs form functional networks with a broad range of effects on DNA methylation, chromatin architecture, transcriptional regulation, post-transcriptional RNA processing (e.g., RNA modifications, quality control and transport), and translation.

MicroRNAs (miRNAs) are an important subclass of ncRNAs that is responsible for regulating the expression of large and evolving functional gene networks through their roles as environmental biosensors (Schratt, 2009). Moreover, miRNAs play key roles in neural differentiation, maintenance, and plasticity (Schratt, 2009). miRNAs are first transcribed as longer primary miRNA (pri-miRNA) transcripts that subsequently form mature ~22 nucleotide molecules. A single miRNA can target large numbers of mRNAs and regulate them through different degrees of sequence-specific interactions. miRNAs bind to their cognate mRNAs in 3' regulatory regions or in specific coding regions repressing translation of these transcripts or sequestering them for storage or degradation.

Fragile X Mental Retardation protein (FMRP) is one of the factors associated with the miRNA pathway, and it is encoded by the FMR1 gene, which is mutated in Fragile X syndrome (FXS), a leading cause of autism spectrum disorders that is associated with an epileptic syndrome suggestive of Rolandic epilepsy in approximately 20% of cases (Hagerman and Stafstrom, 2009). FMRP is an RNA binding protein (RBP) that is involved in post-transcriptional processing and local synaptic translation of a large subset of mRNAs. The actions of FMRP are highly integrated with miRNAs regulatory pathways at many levels through interactions with specific miRNAs and protein effectors of miRNA biogenesis (e.g., Dicer) and function (e.g. RISC complex) (Cheever and Ceman, 2009). Functionally, FMRP plays a key role in modulating synaptic function and plasticity. Intriguingly, glutamatergic and GABAergic synaptic dysfunction at the mRNA and protein level underlie neuronal hyperexcitability in epilepsy associated with Fragile X syndrome (Hagerman and Stafstrom, 2009). These observations suggests that FMRP-mediated synaptic dysfunction in Fragile X syndrome that leads to epilepsy may, in part, be due to deregulated miRNA pathways and impaired coupling

of neuronal excitation and inhibition. Moreover, a recent study showed that tissues from patients with autism spectrum disorders exhibit dysregulation of miRNA expression profiles, and the predicted target genes of these miRNAs include known genetic causes of autism spectrum disorders that are also characterized by epilepsy (e.g., CNTNAP2, GABRB3, FMR1, MECP2, RELN, TSC1, and TSC2) (Abu-Elneel et al., 2008).

Furthermore, a screening of epilepsy patients for mutations in the epileptogenic factor, AP3M2, revealed 21 sequence variations, including 11 in 5' and 3' UTRs and 10 in introns (Huang et al., 2007). One of these 3' UTR single nucleotide polymorphisms (SNPs) was found in patients with generalized epilepsy (GE) and generalized epilepsy with febrile seizures plus (GEFS+) and was located in a binding site for miR-422a suggesting that this sequence variation may disrupt the normal functional relationship between specific miRNA pathways and the AP3M2 mRNA, and in turn lead to epileptogenesis (Huang et al., 2007).

miRNA expression is also dynamically modulated in animal models of epilepsy (Liu et al., 2009; Nudelman et al., 2009). For example, profiles of miRNA expression measured in brain and blood after kainic acid induced seizures are selectively and temporally regulated (Liu et al., 2009). Liu et al. found that 24 hours after kainic acid seizures, 13 and 10 miRNAs were up regulated more than twofold in brain and blood, respectively, and 18 and 21 miRNAs were similarly down regulated in brain and blood, respectively. Differential expression of these miRNAs correlated with differential expression of their target mRNAs, including many implicated in pathways such as cell death, organismal development, cell cycle, cell morphology and gene expression. These results suggest that miRNA networks regulate a spectrum of processes in both the CNS as well as non-neural systems during epileptogenesis, and further that these may serve as novel clinical biomarkers and candidates for targeted therapies for epilepsy.

In addition, after pilocarpine induced seizures, the primary transcript of pri-miR-132 is rapidly and transiently increased and subsequently the mature miR-132 is increased (Nudelman et al., 2009). Intriguingly, miR-132 is highly conserved and enriched in neurons where it plays a role in activity-dependent structural and functional plasticity (Impey et al., 2009; Wayman et al., 2008). Further, miR-132 is regulated by CREB and other redox sensitive factors (Lee et al., 2007), which is important because oxidative stress is implicated in seizure induced neurodegeneration of the hippocampus in the pilocarpine model of epilepsy (Freitas, 2009). In addition, miR-132 is induced by photic entrainment cues via a MAPK/CREB-dependent mechanism, responsible for modulating clock-gene expression, and attenuates the entraining effects of light (Cheng et al., 2007). These miRNA pathways are also linked to epigenetic pathways involved in DNA methylation and chromatin remodeling. In fact, miR-132 regulates the translation of MeCP2 (Klein et al., 2007). Furthermore, miR-132 is itself regulated by REST (Wu and Xie, 2006).

Like miRNAs, long ncRNAs (lncRNAs) represent another important subclass of ncRNAs that may also play a role in epilepsy. lncRNAs are implicated in the regulation of chromatin remodeling, transcription, and post-transcriptional RNA processing (Mercer et al., 2009). Further, aberrant lncRNA expression and function have been associated, directly and indirectly, with an increasing number of diseases (Mercer et al., 2009). For example, FMR4 and ASFMR1 are lncRNAs that are derived from the FMR1 gene locus, which is mutated in Fragile X syndrome (Khalil et al., 2008; Ladd et al., 2007). Like FMR1, both FMR4 and ASFMR1 are silenced in patients with this disorder (Khalil et al., 2008; Ladd et al., 2007). Further, FMR4 exhibits intrinsic anti-apoptotic activity (Khalil et al., 2008). These observations suggest that the functions of these lncRNAs may influence the complex clinical phenotypes of mutations present at this genomic site, including epileptic manifestations.

In addition, lncRNAs play important roles in modulating synaptic and neural network connectivity and plasticity and also in learning and memory (Mattick and Mehler, 2008; Mehler, 2008; Mercer et al., 2008). For example, the brain cytoplasmic RNA 1 (BC1) is a regulatory lncRNA that plays an important role in protein-synthesis-dependent modulation of neuronal excitability (Zhong et al., 2009). BC1 is selectively targeted to somatodendritic domains of neurons where it modulates the initiation of local synaptic translation, and recent studies have demonstrated that BC1 negatively regulates dopamine D2 receptor-mediated synaptic transmission in the striatum and represses the translational stimulation that results from synaptic activation of group I metabotropic glutamate receptors (mGluRs) (Centonze et al., 2007; Zhong et al., 2009). Further, the absence of BC1 leads to neuronal hyperexcitability with significantly elevated group I mGluR-mediated gamma frequency oscillations on cortical electroencephalogram (EEG) recordings and a propensity for convulsive seizures (Zhong et al., 2009). Moreover, the administration of kainic acid transiently increases the level of BC1 through effects on factors that bind to regulatory motifs in the BC1 gene promoter (Kobayashi et al., 2000). Intriguingly, the BC1 gene promoter contains both positive and negative regulatory elements, including a motif similar to but distinct from the neuron-restrictive silencer element (NRSE) that is bound by REST, and BC1 is a binding partner of FMRP.

Evf2 is another lncRNA that may play a role in the pathogenesis of epilepsy because it is important for transcriptional regulation of DLX5 and DLX6, transcription factors responsible for the development of distinct subtypes of GABAergic neurons that mediate the excitability of neural networks in the forebrain (Bond et al., 2009). Evf2 acts through cis- and trans-acting mechanisms including the recruitment of MeCP2 to important Dlx5/6 gene regulatory elements. Evf2 mouse mutants exhibit reduced numbers of GABAergic interneurons in early postnatal hippocampus and dentate gyrus, and reduced synaptic inhibition was observed in these animals suggesting that precise Evf2 gene regulation in the embryonic brain is critical for the proper formation of GABAergic neuronal circuitry in adult brain (Bond et al., 2009). Thus, Evf2 mediates the structure and function of GABAergic neuronal networks that in turn are responsible for modulating the neural network excitability that is important in epilepsy.

RNA editing

RNA editing is an epigenetic mechanism that is responsible for RNA post-transcriptional processing (Mehler and Mattick, 2007). It promotes the generation of significant molecular diversity and environmental plasticity in transcripts through directed modification of nucleotides in RNA molecules. Editing of mRNA transcripts can change amino acid coding potential and protein function. A family of enzymes called adenosine deaminases that act on RNA (ADARs) is responsible for catalyzing adenosine to inosine (A to I) editing events. These enzymes are exquisitely environmentally responsive and preferentially active in the brain where they edit transcripts involved in critical neuronal processes. In fact, A to I RNA editing was initially described in mRNAs encoding ion channels and neurotransmitter receptors that mediate neuronal excitability. It serves as a mechanism for fine-tuning gating, permeability, kinetics, intracellular trafficking and assembly of these factors. Subsequent studies established that the majority of RNA editing occurs in untranslated regions of protein-coding transcripts and in ncRNA transcripts, where editing events may regulate transport, stability, further processing and metabolism of these RNAs (Athanasiadis et al., 2004; Blow et al., 2004; Li et al., 2009). For example, editing of miRNA transcripts can alter miRNA-target mRNA interactions (Blow et al., 2006). In addition to adenosine to A to I editing, (deoxy)cytidine to (deoxy)uridine ([d]C to [d]U) nucleotide editing is catalyzed by the apolipoprotein B (ApoB) editing catalytic subunit (APOBEC) family of enzymes, which are cytidine deaminases that act on both RNA as well as DNA molecules (Bransteitter et al., 2009). Accordingly, abnormalities in RNA editing are implicated in a range of CNS disorders including stroke, amyotrophic lateral sclerosis, schizophrenia, and depression (Mehler, 2008).

Abnormal profiles of RNA editing are implicated in human epilepsy and in animals with epilepsy. For example, A to I editing of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor subunit GluR2 at the R/G site is significantly increased in hippocampal tissue from temporal lobe epilepsy patients (Vollmar et al., 2004). This editing event increases the permeability of glutamate receptors for calcium ions, which promotes excitotoxicity and causes severe neuronal dysfunction (Vollmar et al., 2004). Similarly, APOBEC-mediated C to U editing of the glycine receptor subunits, GLRA2 and GLRA3, is increased in hippocampal tissue from temporal lobe epilepsy patients, and the level of editing correlates with the frequency of generalized tonic-clonic seizures and the degree of hippocampal sclerosis (Eichler et al., 2008; Meier et al., 2005).

Moreover, transgenic mice with aberrant profiles of GluR2 mRNA editing at the Q/R site at position 586 have glutamate receptors with abnormal permeability to calcium, and these animals suffer from severe epilepsy and die within two weeks of age (Brusa et al., 1995). Similarly, knockout mice that lack the ADAR2 editing enzyme suffer from severe epilepsy and die within three weeks of birth because of underediting of the GluR2 Q/R site. In fact, the epileptic phenotype of these mice can be rescued by the introduction of a properly edited version of GluR2 (Higuchi et al., 2000).

In addition, BRUNOL4 is an RBP that has roles in mediating aspects of RNA post-transcriptional processing, including RNA editing, exhibits high levels of expression in the hippocampus, and is implicated in epilepsy. A transgenic mouse with a mutation in the gene encoding BRUNOL4 displays a complex epileptic phenotype characterized by a reduced seizure threshold and multiple seizure types including severe tonic-clonic, limbic, and manifestations of absence seizures (Yang et al., 2007). Gene expression analysis of these animals revealed down regulation of genes involved in neuronal excitability in the hippocampus (Yang et al., 2007).

EPIGENETIC MEDICINE

Drugs that target pathways involved in DNA methylation and histone modifications are already available and additional epigenetic pharmacological agents are actively under development for the treatment of various disease states (Szyf, 2009). The most well characterized drugs that affect DNA methylation are 5-azacytidine (5-azaC), 5-aza-2-deoxycytidine (5-azaCdR or decitabine) and zebularine, which act as analogs of the nucleoside cytosine and non-specifically inhibit the function of DNA methyltransferase enzymes. The effects of these agents on epileptogenesis are not well characterized (Becq et al., 2005). Additional epigenetic therapies include histone deacetylase enzyme inhibitors, such as sodium butyrate, trichostatin A and valproic acid, which have the potential to reprogram gene expression profiles in epileptic disorders and possibly even reverse the process of epileptogenesis. For example, treatment with sodium butyrate increases histone H3 and H4 acetylation in the hippocampus and cerebral cortex of mice and modulates the abilities of dizocilpine and flurazepam to antagonize electrically precipitated seizures (Deutsch et al., 2009; Deutsch et al., 2008). Also, chronic administration of valproic acid results in increased histone H3 acetylation in the brain (Eleuteri et al., 2009). In addition, treatment with valproic acid dramatically accelerates RELN and GAD promoter demethylation, providing evidence that histone deacetylase inhibitors like valproic acid can directly or indirectly facilitate DNA demethylation and simultaneously modify multiple aspects of the epigenome (Dong et al., 2007). Intriguingly, kainic acid induced seizures promote the proliferation of neural progenitor cells in the DG and are also associated with long term cognitive impairment, and valproic acid blocks this aberrant seizure induced neurogenesis through its ability to inhibit histone deacetylases and to alter histone deacetylase-dependent gene expression within the DG (Jessberger et al., 2007). Further, this inhibition of neurogenesis protects the animals from seizure induced cognitive impairments suggesting that

that the effectiveness of valproic acid as an antiepileptic drug may be partially explained by the histone deacetylase-dependent inhibition of aberrant neurogenesis induced by seizure activity within the adult hippocampus (Jessberger et al., 2007).

Moreover, in a rat-kindling model of temporal lobe epilepsy, the glycolytic inhibitor 2-deoxy-D-glucose (2DG) reduces kindling and abrogates seizure-induced increases in BDNF and its receptor, TrkB, by modulating REST mediated recruitment of the transcriptional coregulator, CtBP, to the BDNF promoter (Garriga-Canut et al., 2006). Intriguingly, this observation suggests that anti-glycolytic compounds may represent drugs for treating epilepsy and further that the mechanism of action of these agents may include direct effects on metabolic parameters as well as indirect effects on chromatin state.

PERSPECTIVES

Recent studies have significantly advanced our understanding of the genetic bases of epilepsy and the molecular and cellular mechanisms underlying the process of epileptogenesis. However myriad questions remain regarding the onset, progression, and optimal therapies for the panoply of pediatric and adult epileptic syndromes and their associated co-morbidities. There are currently no preventative or disease modifying treatments available for epilepsy, and symptomatic therapies only offer a limited range of clinical efficacy.

In this review, we highlighted emerging evidence that is beginning to elucidate the roles played by epigenetic factors in various epilepsy syndromes and also in heralding epileptogenesis. Firstly, a number of genes that cause epilepsy when mutated in humans encode factors associated with DNA methylation (e.g., MeCP2), histone code modifications and chromatin remodeling (e.g., ATRX, KDM5C, NSD1, and PRICKLE1) and ncRNA regulation (e.g., FMRP). Genes that cause epilepsy in animal models also encode factors involved in histone code modifications and chromatin remodeling (e.g., HDAC4 and PIM3) and RNA editing (e.g., ADAR2 and BRUNOL4). In addition, epigenomic profiling in tissues from humans and animals with epilepsy shows abnormal patterns of DNA methylation, histone code modifications, ncRNA expression, and/or RNA editing that are genome-wide and more selective for factors implicated in various aspects of epileptogenesis including neurotransmitter receptors (e.g., GluR2, GLRA2, and GLRA3), growth factors (e.g., BDNF), extracellular matrix (ECM) proteins (e.g., RELN), and transcriptional regulators (e.g., CREB, c-fos, and c-jun). Lastly, pharmacological or genetic manipulations of epigenetic pathways demonstrate the potential to ameliorate epilepsy and even to mitigate the process of epileptogenesis. In fact, valproic acid is a commonly used broad-spectrum anti-epileptic drug (AED) that has multiple cellular effects, which include the ability to inhibit voltage-dependent sodium currents and a relevant epigenetic mechanism of action (i.e., inhibition of histone deacetylase enzymes) that is also implicated in its clinical efficacy.

These observations provide compelling evidence for the conclusion that epileptic disorders are characterized not only by genetic predisposition and environmental insults but also by a spectrum of dysfunctional epigenetic processes, which serve as the molecular arbiters for integrating the effects of inherited and acquired etiological factors and thus for modulating the clinical manifestations of a continuum of epileptic disorders. This view of the role played by epigenetic mechanisms in the pathobiology epilepsy is similar to that which is emerging for a broad range of neurological and neuropsychiatric disorders, including those with overlapping pathological features at molecular, cellular and network levels and clinical co-morbidities (e.g., cognitive and behavioral). Therefore, future studies of epigenetic mechanisms represent an important and promising means for identifying novel disease biomarkers, for developing a more biologically based nosology for epileptic disorders, as well as for designing rational treatment strategies that overcome the serious limitations of current therapeutic approaches.

Indeed, targeted epigenetic reprogramming offers a potential strategy for halting the process of epileptogenesis and non-reductionist approach for remodeling neural networks whose balance between excitation and inhibition is dysfunctional in epilepsy. Epigenetic processes are responsible for dynamically regulating the expression and function of genes (e.g., different classes of ion channels and receptors) and gene networks that govern the intrinsic excitability and synaptic connectivity of individual neurons and of neural networks and mediate the compensatory changes in neuronal properties and neural network performance which occur throughout life (Marder and Goaillard, 2006). Thus, epigenetic reprogramming of homeostatic mechanisms in the CNS can orchestrate diverse changes that promote and maintain neural network stability.

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