CHAPTER

Epigenetic mechanisms in epilepsy

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Abstract

In humans, genomic DNA is organized in 23 chromosome pairs coding for roughly 25,000 genes. Not all of them are active at all times. During development, a broad range of different cell types needs to be generated in a highly ordered and reproducible manner, requiring selective gene expression programs. Epigenetics can be regarded as the information management system that is able to index or bookmark distinct regions in our genome to regulate the readout of DNA. It further comprises the molecular memory of any given cell, allowing it to store information of previously experienced external (e.g., environmental) or internal (e.g., developmental) stimuli, to learn from this experience and to respond. The underlying epigenetic mechanisms can be synergistic, antagonistic, or mutually exclusive and their large variety combined with the variability and interdependence is thought to provide the molecular basis for any phenotypic variation in physiological and pathological conditions. Thus, widespread reconfiguration of the epigenome is not only a key feature of neurodevelopment, brain maturation, and adult brain function but also disease.

Keywords

gene regulation, DNA methylation, histone code, noncoding RNA, chromatin remodeling, temporal lobe epilepsy, hippocampus, genetic epilepsy, epileptic encephalopathy, metabolism

1 "BOOKMARKING" THE GENOME

The term epigenetics summarizes alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (beyond a simple "ON" and "OFF") without changes in DNA sequence. Liberally, epigenetic mechanisms include DNA methylation, posttranslational histone-tail modifications, selective utilization of histone variants (e.g., H2A.X, H2A.Z, H3.3), ATP-dependent chromatin remodeling processes, and action of noncoding RNAs (ncRNAs).

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Whether an epigenetic tag should be meiotically and/or mitotically heritable remains a matter of debate. Three major steps contribute to epigenetic gene regulation: (1) writing and/or erasing tags on the chromatin template by specific enzymes, which mark genes for active transcription or silencing, (2) reading the tags using proteins containing specialized recognition modules, and (3) recruitment of additional enzymes and proteins or specific ncRNAs that either initiate fine tune or terminate gene expression. Despite an obvious implication in developmental processes epigenetics also comprises the molecular memory of any given cell, allowing it to store information of previously experienced external (e.g., environmental) or internal (e.g., developmental) stimuli, to learn from this experience and to respond. Epigenetic mechanisms can be synergistic, antagonistic, or mutually exclusive and their large variety combined with the variability and interdependence is thought to provide the molecular basis for any phenotypic variation in physiological and pathological conditions. In epilepsy research, this is especially interesting with regard to the stimulus-driven activity and connectivity of postmitotic neurons and glia in the adult brain.

2 CHROMATIN STRUCTURE

Understanding epigenetics requires basic understanding of chromatin structure and organization. The building blocks of chromatin are DNA and protein, and the smallest organizational unit is the nucleosome, which consists of 147 bp of double-stranded DNA wrapped around octamers of histone proteins (two copies of each "core" histone, i.e., H2A, H2B, H3, and H4). Most chromatin in mammalian cells exists in a condensed and transcriptionally silent form, but regions harboring actively transcribed genes are less condensed in their structure allowing interaction with the transcription machinery and other regulatory proteins/protein complexes. Histones and DNA are chemically modified with epigenetic marks which influence chromatin structure either by altering the electrostatic interaction of DNA and histones or the affinity of specific binding proteins. The following section will give an overview on the main epigenetic mechanisms, their implication in brain development and function, as well as their role in the pathogenesis of genetic and symptomatic epilepsy, and provide a short overview about novel and promising therapeutic strategies that possibly derive from this knowledge in the future.

3 DNA METHYLATION: STRATEGY FOR TRANSCRIPTIONAL SILENCING

DNA methylation is a covalent chromatin modification, fuelled by the transmethylation pathway, where a methyl group ($-CH_3$) is transferred from *S*-adenosyl methionine (SAM) to the 5′ position of cytosine nucleotides. Thereby, SAM is converted to *S*-adenosyl homocysteine (SAH) and further hydrolyzed to adenosine and homocysteine (HCY). Adenosine is cleared by adenosine kinase-mediated phosphorylation to AMP, while HCY is converted to methionine in a folate-dependent manner (Boison et al., 2013).

It has been estimated that 2–7% (depending on species) of the total cytosine in mammalian DNA is methylated (Razin and Riggs, 1980). The methylation of DNA is mediated by the members of the DNA methyltransferase (DNMT) family, conventionally classified as *de novo* (DNMT3A and DNMT3B) and *maintenance* (DNMT1) (Bestor, 2000; Goll and Bestor, 2005). While DNMT1 shows a preference for hemimethylated DNA, DNMT3A, and 3B do not depend on, and in fact do not even recognize, the methylation status of their target DNA (Gowher and Jeltsch, 2001). Following DNA methylation, recruitment of "reader proteins," namely, the methyl-CpG-binding domain (MBD) family-containing proteins such as MBD1, MBD2, MBD3, MBD4, MeCP2, and KAISO, is necessary to mediate downstream effects (Fournier et al., 2012).

In all higher eukaryotes including humans, DNA methylation is mainly confined to CpG dinucleotides (Dulac, 2010), but non-CpG methylation (i.e., CpNpG, CpNpN) has also been reported for embryonic stem cells, induced pluripotent stem cells, oocytes, and the brain, most prominently at CpA and to a lesser extent at CpT or CpC dinucleotides (Barres et al., 2009; Clark et al., 1995; Grandjean et al., 2007; Ichiyanagi et al., 2013; Lister et al., 2009, 2013; Varley et al., 2013). Although present throughout the entire genome, non-CpG methylation is particularly enriched at certain genomic features (i.e., exons, introns, 3′ untranslated region) and has been correlated with increased gene expression (Lister et al., 2009). Knock-out studies as well as *in vitro* studies analyzing methylation kinetics provide evidence that non-CpG methylation is mediated by *de novo* DNMTs (Gowher and Jeltsch, 2001; Ziller et al., 2011). The biological impact of non-CpG methylation so far remains poorly understood. However, as this mark is highly present in the adult mouse and human brain, but rare or absent in other differentiated cell types, a unique role in mammalian brain development and function can be assumed. Accumulation and positional conservation of non-CpG methylation in neurons, but not glia, makes it the dominant form of methylation in the human neuronal genome and points at a role in neural lineage commitment. Furthermore, high intragenic non-CpG methylation seems to specifically mark genes that escape X-chromosome inactivation (Lister et al., 2013).

Approximately 70% of human genes are linked to promoter CpG islands, whereas the remaining promoters tend to be depleted in CpGs. The great majority of CpG islands is unmethylated at all stages of development in all normal, non-diseased tissue types (Bird, 2002; Edwards et al., 2010), thereby, retaining an open chromatin structure for accession of transcription factors and dynamic regulation of gene expression (Bergman and Cedar, 2013). When present, promoter methylation is frequently correlated with gene repression, whereas gene body methylation shows less stringent associations with gene silencing. There are reports that intragenic methylation is indicative for active transcription including the regulation of alternative splicing (Sati et al., 2012). Other studies suggested a role of intragenic DNA methylation in controlling alternative promoter usage, particularly in the brain (Maunakea et al., 2010). It is generally anticipated that downstream effects of DNA methylation may result from (1) interference with transcription factor binding, (2) recruitment of methyl-binding proteins and their associated regulatory

complexes, and/or (3) induced chromatin remodeling. Interestingly, mammalian transcription factor binding sites are more GC rich than the bulk genome and many contain CpGs within their recognition sequence (Deaton and Bird, 2011), suggesting a strong interdependence.

DNA methylation is implicated in regulation of gene transcription, silencing of repetitive DNA elements, and genomic imprinting (Edwards and Ferguson-Smith, 2007). It could clearly be associated with X-chromosome inactivation in females (Minkovsky et al., 2012), aging (Madrigano et al., 2012; Numata et al., 2012), lineage commitment, e.g., during neurogenesis (Ma et al., 2010), and in neural plasticity in the developing and adult brain (Feng et al., 2010; Guo et al., 2011; Levenson et al., 2006; Miller and Sweatt, 2007; Nelson et al., 2008; Reik, 2007; Wu and Zhang, 2010). Genomic- or locus-dependent DNA methylation loss was observed in both physiologic and pathologic conditions. DNA methylation may be *passively* lost or "diluted" during cell cycle, when DNA methylation enzymes and/or their complexes are denied access to the newly replicated DNA. In addition, *active* DNA *de*methylation may be facilitated, also in nondividing postmitotic cells (Gong and Zhu, 2011). A common intermediate in the process of active DNA demethylation seems to be 5-hydroxymethylcytosine (5-hmC), and the enzyme promoting 5-mC oxidation to 5-hmC is the ten-eleven translocation (TET) family of methylcytosine dioxygenases. Function of 5-hmC is still matter of debate, but there is some evidence for 5-hmC-mediated epigenetic dynamics during postnatal development and aging, also in the human brain (Hahn et al., 2013; Szulwach et al., 2011; Wang et al., 2012). It could be shown that transcriptional activity is associated with intragenic enrichment of hydroxymethylation. While cell type-specific genes show conserved 5-hmC patterns in the fetal and adult brain, loss of 5-hmC is associated with transcriptional downregulation during development (Lister et al., 2013). 5-hmC is involved in higher order brain function, given that Tet1 knock-out animals show no difference in brain size or morphology, but significant reduction in 5-hmC levels in the cortex and hippocampus accompanied with abnormal hippocampal long-term depression and impaired memory extinction (Rudenko et al., 2013).

Improper establishment, maintenance, or recognition of methylation marks has been described in cancer, imprinting disorders, repeat instability disorders and those that result from defects in "writing," "reading," and "erasing" DNA methylation. Rett syndrome and related neurodevelopmental disorders belong to the latter as they result from defects in the methyl-CpG-binding protein 2 (MeCP2) machinery. Typical Rett features can be mimicked in a mouse model lacking Mecp2. In contrast, mice overexpressing Mecp2 present normal until 10–12 weeks of age, but then show neurological symptoms, such as seizures, forepaw clasping, hypoactivity, and spasticity. Later seizures become more frequent and animals die prematurely, which indicates that levels of Mecp2 in the central nervous system (CNS) are tightly regulated and crucial for proper brain function. Clinical manifestations of Rett syndrome such as mental retardation, seizures, muscular hypotonia, and acquired microcephaly may result from aberrant expression of imprinted target genes that escape proper

regulation upon the loss of function of Mecp2, e.g., ubiquitin protein ligase E3A (Ube3a) or distal-less homeobox 5 (Dlx5; Bienvenu and Chelly, 2006). What makes MeCP2 even more important is that it also has been identified as the major 5-hmC-binding protein in the brain with similar high binding affinities for 5-hmC as for 5-mC (Mellen et al., 2012). So far, no data are available about 5-hmC in epilepsy- or seizure-associated neurodevelopmental diseases. But DNA hydroxymethylation is important in regulating gene expression in the aging brain and is broadly altered in postmortem brains of patients with Alzheimer's disease (Chouliaras et al., 2013).

4 HISTONE MODIFICATIONS: DETERMINANTS OF ACCESSIBILITY

Posttranslational histone modifications include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation of specific amino acids in the N-terminal tail of histones (Khorasanizadeh, 2004). Some modifications may activate gene expression, while others work in the opposite direction. Even the same modification can have opposing effects in a dose-dependent manner. Furthermore, complexity is offered by the ordered and sequential nature of histone modifications that either attenuate or accentuate transcription (Fischle, 2008; Wang et al., 2008). Through this complexity the so-called "histone code" ensures nuclear processes (transcription, replication, DNA-damage response) to be directed to the required region of the genome at appropriate time mediating unique cellular responses and biological outcomes. But this same complexity is what makes interpretation of histone marks difficult.

In histone acetylation, a negatively charged acetyl group is added to Lysine (K) residues on histone proteins. Neutralizing the positive charge on histone proteins interferes with the usual electrostatic affinity between histones and negatively charged DNA backbone, which is thought to render chromatin more accessible for recruitment of non-histone transcriptional regulatory proteins promoting gene expression (Graff and Tsai, 2013). There are 26 sites of acetylation on a nucleosome and histone acetylation is dynamically regulated by histone acetyltransferases (HATs) and the antagonistic effects of histone deacetylases (HDACs) to achieve appropriate levels of transcription. Intriguingly, many proteins initially characterized as being involved in transcriptional regulation (transcription factors, corepressors, and coactivators) were later identified to possess HAT or HDAC activity (e.g., cAMP-response element-binding protein, CREB; CREB-binding protein, CBP/P300; P300/CBP-associated factor; TATA-binding protein-associated factor II, TAF-II; RE1-Silencing Transcription Factor, REST; nuclear factor kappa B, NFκB). However, many acetylases and deacetylases that have been identified to modify histone substrates *in vitro* are also known to target non-histone proteins, making an interpretation of the predominant physiological role of these enzymes difficult (Khan and Khan, 2010).

Histone methylation can target Lysine or Arginine residues of histone tails, may alter gene expression in both directions and has been associated with different stages of transcriptional control, mRNA splicing, DNA repair, and replication (Di Lorenzo and Bedford, 2011; Lachner and Jenuwein, 2002). All histones have the capability to be methylated on one or more residues, but some residues seem to be targeted more frequently than others. Methylation of H3K9, H3K27, H4K20 has been associated with condensed chromatin states and gene repression (Martin and Zhang, 2005). However, the degree of methylation at these sites may vary and cause different effects (Barski et al., 2007). H3K9 methylation has been particularly implicated in the induction and propagation of heterochromatin formation, whereas H4K20 methylation is a key regulator of biological processes that ensure genome integrity, such as DNA-damage repair, DNA replication, and chromatin compaction during cell cycle (Jørgensen et al., 2013; Tardat et al., 2007). H3K27 methylation recruits PRC1, which in turn compacts the targeted chromatin and contributes to the inactivation of gene expression. Given the critical role of H3K27 methylation in the balance of gene activity, it is not surprising to find anomalies of this system in different types of cancer (Martinez-Garcia and Licht, 2010). Both H3K9- and H3K27-trimethylation have been further described to play a crucial role in regulating DNA methylation, thereby linking different epigenetic repression systems (Lehnertz et al., 2003; Vire et al., 2006). This interdependence seems to hold true for most of the genome except CpG islands in differentiated cells (e.g., neurons), where there is more recent evidence that H3K27-trimethylation and DNA methylation are mutually exclusive and work antagonistically to silence genes (Brinkman et al., 2012). Other methylation marks seem to be restricted to active gene promoters, e.g., H3K4 methylation was associated with transcription initiation, while H3K36 methylation may support transcription elongation (Rando, 2012). Monomethylation of H3K4 distinguishes active enhancers, whereas trimethylation of H3K4 is highly enriched at promoters and around transcriptional start sites (Heintzman et al., 2007). Binding of H3K4-specific methyltransferases protects promoters of developmental genes from DNA methylation (Smith and Meissner, 2013) and H3K4 dimethylation inhibits deacetylation by precluding HDAC recruitment, further undermining the role of H3K4 methylation in gene activation (Bernstein et al., 2002). Intriguingly, developmentally critical genes frequently contain a bivalent domain within their promoters, where functionally opposing H3K4 and H3K27 trimethylation marks are present at the same time. This pattern allows lineage-specific genes to be either silenced or activated as differentiation proceeds (Bernstein et al., 2006). The dynamics of histone-lysine methylation are dependent on the antagonistic actions of methyltransferases and demethylases in a fashion similar to acetylation. However, a comparison of the turnover rates of histone-lysine acetylation (which has a half-life of 2–40 min) and methylation (which has a half-life of 0.3–4 days) shows that the events occur at different timescales with a much slower methylation turnover (Hojfeldt et al., 2013).

In addition to the well-known acetylation or methylation, histones can also be ubiquitinated (Zhang, 2003). This modification was long only associated with the

protein degradation system, whereas its contribution to transcriptional regulation remained uncertain. Attachment of polyubiquitin chains (four or more ubiquitin moieties attached to each other) seems to be the mechanism used to target proteins for degradation. In contrast, monoubiquitination was suggested to play diverse roles in processes such as DNA repair, protein trafficking, and transcription. It has further been demonstrated that monoubiquitination at multiple sites of the same protein can also target proteins for proteasomal degradation (Metzger et al., 2014). Proteasome-dependent degradation of transcription factors or linker protein H1 is important regulatory mechanisms, but now also proteasome-independent mechanisms have come into focus. The TATA-binding protein (TBP)-associated factor, TAF-II250, is a component of the general transcription factor TFIID and was described to ubiquitinate linker histone H1, which seems to promote gene activation (Muratani and Tansey, 2003). Histone H2A ubiquitination could be linked to X-chromosome inactivation and polycomb (PcG) silencing (de Napoles et al., 2004; Wang et al., 2004). Furthermore, the interdependence of H2A ubiquitination and histone as well as DNA methylation could be established (Wu et al., 2008). Ubiquitination of H2B on Lysine K123 is mediated by the ubiquitin conjugating enzyme E2A (UBE2A) and has been suggested to be implicated in the maintenance of telomeric gene silencing by regulating histone H3K4 and H3K79 methylation, however, through an unknown mechanism (Muratani and Tansey, 2003). This interrelation has first been established in yeast, but could also be proven in human cells (Kim et al., 2009). The presented findings prove once more that the cooperation among different epigenetic modifications plays an important role in transcriptional regulation.

Perturbations of the histone code may contribute to seizure generation. In experimental epilepsy localized and global changes in histone acetylation have been described (Huang et al., 2002; Sng et al., 2005, 2006; Tsankova et al., 2004). Evidence for an implication of histone methylation in epilepsy comes from patients with genetic defects targeting histone methyltransferases, e.g., enhancer of zeste homolog 2 (EZH2; Weaver syndrome), euchromatic histone-lysine *N*-methyltransferase 1 (EHMT1) and lysine (K)-specific methyltransferase 2C (KMT2C; Kleefstra syndrome), MLL (Wiedemann–Steiner syndrome). Mutations targeting ubiquitin ligase UBE3A affect about 5–10% of patients with Angelman syndrome. However, there is no evidence yet that UBE3A may be involved in histone ubiquitination and regulation of gene expression.

5 ncRNAS: NO LONGER JUNK

ncRNAs are small functional RNA molecules that function directly as structural, catalytic, or regulatory molecules rather than serving as templates for protein synthesis. They include small ncRNAs like siRNAs, micro-RNAs (He and Hannon, 2004) and PIWI-interacting RNAs (Luteijn and Ketting, 2013), as well as long ncRNAs (Mercer et al., 2009), all characterized by individual biogenesis and maturation pathways, posttranscriptional processing, conformational changes, intra- as well

as intercellular trafficking, modes of target recognition, regulatory properties, and transgenerational epigenetic states (Qureshi and Mehler, 2012). The role of ncRNAs has long been neglected, but we are becoming more and more aware that the variety of ncRNAs, their dynamics of action, and versatile regulatory potential actually correlate with the enormous complexity, e.g., of the CNS (Cao et al., 2006). Here, we will focus on micro-RNAs and long ncRNAs that have been implicated in epileptogenesis and chronic epilepsy.

5.1 SMALL ncRNAs

Micro-RNAs are a group of highly conserved small regulatory RNAs with an approximate length of ~22 nt, essential for the correct function of the nervous system, which shows the broadest spectrum of miRNA expression of all human tissues (Esteller, 2011). An estimated 70% of all micro-RNAs is expressed in the brain, with a major role in development and neuronal function including proliferation of neural stem and progenitor cells, neuronal differentiation and maturation (De Pietri Tonelli et al., 2008; Sun et al., 2013), neurite outgrowth, and synaptogenesis (Jovicic et al., 2013; Olde Loohuis et al., 2012; Yoo et al., 2011). Evidence for global effects of micro-RNAs on CNS development derived from studies on region specific genetic ablation of *Dicer* and subsequent blocking of micro-RNA biogenesis at different developing stages, which resulted in brain malformation, reduced neural progenitor pool, and abnormal neuronal differentiation (Davis et al., 2008; Kawase-Koga et al., 2009). Similarly, knockout of specific miR genes, e.g., miR-9, miR-124, proved their implication in embryonic and adult neurogenesis (Sun et al., 2013). Some studies further supported a locally restricted mode of action for micro-RNAs in, e.g., specific cell types or even subcellular compartments, where they modulate synaptic activity and neuronal connectivity (Hengst et al., 2006; Schratt et al., 2006). Generally, mature micro-RNAs are able to mediate gene repression by either destabilization of mRNA transcripts (i.e., decapping, deadenylation, degradation) or inhibition of mRNA translation depending on complementarity to the target sequence. More recently, there is also evidence for transcriptional and translational activation by micro-RNAs introducing a new level of complexity (Vasudevan et al., 2007).

In experimental models of status epilepticus and in human epilepsy select changes to micro-RNA expression within the brain have been identified (Aronica et al., 2010; Jimenez-Mateos et al., 2012). Knockdown of miR-134 or miR-34a was neuroprotective, reduced seizure severity frequency of spontaneous recurrent seizures. Persistent overexpression of miR-146a in reactive astrocytes in human epileptic hippocampus supports a possible involvement in the astroglial inflammatory response occurring in temporal lobe epilepsy (TLE). MiR-132, another micro-RNA overexpressed in TLE, is further thought to influence neuronal morphology, hyperexcitability, and via regulation of its target gene MeCP2 possibly also influences cognitive dysfunction (Henshall, 2013; Peng et al., 2013). All studies promote miRNAs as novel therapeutic targets to, e.g., reduce brain injury, pro-epileptogenic inflammatory signalling, and/or functional changes of neurons.

5.2 LONG ncRNAs

In contrast to the small ncRNAs, which are highly conserved and involved in transcriptional and posttranscriptional gene silencing through specific base pairing with their targets, long ncRNAs are poorly conserved at the primary sequence level and only a small number have been functionally well characterized to date. Long ncRNAs were initially thought to be spurious transcriptional noise resulting from low-RNA polymerase fidelity, but their spatiotemporal expression patterns in development (e.g., large numbers are specifically expressed during embryonic stem cell differentiation and in the brain), the identification of promoter structures with conventional chromatin signatures in noncoding loci together with the frequent binding of transcription factors strongly suggest that expression of long ncRNAs is under precise control (Fatica and Bozzoni, 2014; Rinn and Chang, 2012). Long ncRNAs can be expressed from either or both DNA strands. They are often spliced and, in contrast to most mRNAs and micro-RNAs, which ultimately localize to the cytoplasm after processing, long ncRNAs are frequently, but not exclusively, localized in the nucleus. There are many ways of how long ncRNAs facilitate their regulatory properties. Imprinting and selective expression has been described, as well as downstream repression of imprinted genes in a tissue- and allele-specific manner. Thereby, long ncRNAs serve as molecular signals and may act as markers of functionally significant biological events. It has to be noted that long ncRNAs can either act locally on neighboring genes (i.e., in *cis*) or globally on distantly located genes (i.e., in *trans*) (Fatica and Bozzoni, 2014).

Different modes of action have been previously described. Long ncRNAs can function through binding to and altering the activity of, e.g., transcription factors. This is sometimes described as the "decoy" function of long ncRNAs: binding to and titrating away transcriptional activators or repressors or other regulatory proteins. The broader the functions of the transcription factor the "longer" the arm of the ncRNA (Wang et al., 2011). One critical function of ncRNAs is their ability to interact with specific regulatory proteins, serving either as scaffold for protein complex formation or as molecular guide to recruit proteins to a specific target sequence. Many of the long ncRNA-associated proteins appear to be chromatin-modifying factors, e.g., DNMT3B, euchromatic histone-lysine *N*-methyltransferase 2 (EHMT2), KMT2A, lysine (K)-specific demethylase 1A (LSD1)-CoREST, and polycomb repressor complexes (PRC1/2), suggesting a critical role in epigenetic gene regulation (Khalil et al., 2009; Rinn and Chang, 2012).

Long ncRNAs have been implicated in a variety of neurological disorders including epilepsy and others with an associated seizure phenotype (Knauss and Sun, 2013). Endogenous antisense long ncRNA transcripts, which frequently repress their sense-strand protein-coding partners, seem to serve a specific role here. The long noncoding antisense transcript silences the paternal allele of Ube3a and, as this, is involved in Prader–Willi and Angelman syndromes (Meng et al., 2012). BDNF-AS is a long ncRNA that serves as a direct negative regulator of the BDNF gene. In human epileptic neocortex, BDNF-AS is downregulated, while, inversely, its *cis*-antisense partner BDNF is highly upregulated by seizure activity in both animal

models and human epilepsy (Lipovich et al., 2012). This may be regarded as a promising finding with respect to novel treatment strategies, as BDNF serves an instructive role in the development and progression of TLE. Another long ncRNA, FMR1 antisense RNA 1 (FMR1-AS1), is silenced in fragile X patients as well as upregulated in premutation carriers and, although not a direct regulatory transcript for FMR1, has been suggested to contribute to clinical aspects of fragile X syndrome (Khalil et al., 2008).

6 EPIGENETICS IN CNS DEVELOPMENT AND HIGHER ORDER BRAIN FUNCTION

Developmental changes in epigenetic states allow for structural and functional organization of the brain through control of neuro- and gliogenesis, and activity-dependent synaptic plasticity. Epigenetic perturbations together with improper regulation of neurodevelopmental steps can lead to a variety of pathologies including alterations in neurogenesis, aberrant neuronal migration and structural changes of individual cells, and/or large networks, all of which may contribute to the formation of hyperexcitable circuits and seizure activity.

Neurogenesis is defined as the generation of new functional neurons. We distinguish embryonic neurogenesis, which forms the CNS, from adult neurogenesis, which continues at low levels in postnatal and adult brains (Jobe et al., 2012). Epigenetic mechanisms carry out diverse roles in regulating specific aspects of embryonic and adult neurogenesis including stem cell renewal, neuronal fate specification as well as maturation and integration (Ma et al., 2010). DNA-methylating enzymes seem to be involved in neurogenesis, neuronal maturation, and cell survival. Conditional mutant mice targeting Dnmt1 display severe neuronal cell death between E14.5 and 3 weeks postnatally accompanied with striking cortical and hippocampal degeneration (Hutnick et al., 2009). Homozygous deletions of Dnmt1, Dnmt3a, or Dnmt3b in mice are not viable underlining the overall physiological importance of DNA methylation (Li et al., 1992; Okano et al., 1999). The PcG and trithorax group proteins comprise protein complexes with epigenetic function implicated in neural lineage commitment and cellular memory. The specific relevance of the PcG system in neurogenesis and CNS development is exemplified by the neuronal defects in various PcG mouse mutants (Fasano et al., 2009). In mice, genetic ablation of components of the PRC1 (e.g., PcG ring finger oncogene; ring finger protein 2) interferes with cerebral neural stem cell renewal, resulting in progressive postnatal growth retardation and neurological abnormalities manifested, e.g., by an ataxic gait and sporadic seizures (Molofsky et al., 2003; van der Lugt et al., 1994).

Beside brain development, epigenetic modifications have been implicated in higher order brain functions. Histone acetylation is essential to learning and memory, as lack of this modification has been causally related to cognitive impairment in neurodevelopmental disorders, neurodegeneration, and aging (Graff and Tsai, 2013; Peleg et al., 2010). Environmental enrichment as well as treatment with HDAC

inhibitors reestablished histone-tail acetylation, increased dendritic sprouting and number of synapses, as well as reinstated learning behaviour and access to long-term memories in mice (Fischer et al., 2007; Peleg et al., 2010). In humans, mutations of the Creb-binding protein (CREBBP) gene cause Rubinstein–Taybi syndrome. CREBBP is a coactivator of transcription possessing intrinsic HAT activity. In transgenic mouse models of Rubinstein–Taybi syndrome, the HAT activity of Crebbp was recognized as the critical component of memory consolidation (Feng et al., 2007). Histone methylation is also involved in learning and memory. Conditional knockout of histone-methylating enzymes (e.g., Ehmt2; Kmt2d) in adult mice forebrain or hippocampus significantly altered histone methylation and corresponding gene expression changes, resulting in complex behavioral abnormalities and learning impairment (Gupta-Agarwal et al., 2012; Kerimoglu et al., 2013). Evidence for DNA methylation in memory comes from studies showing that functional inhibition of Dnmts blocks hippocampus-dependent memory formation as well as memory consolidation together with deregulated expression of genes known to contribute to synaptic plasticity (Feng et al., 2010; Miller and Sweatt, 2007). Conditional knockout of *maintenance* and *de novo* Dnmts together, but not alone, impairs hippocampal long-term potentiation providing evidence that Dnmt3a and Dnmt1 play redundant roles in regulating learning and memory (Feng et al., 2010). In contrast, genetic deletion of growth arrest and DNA-damage-inducible beta (Gadd45b), a regulator of active DNA demethylation, enhances long-term memory and synaptic plasticity (Sultan et al., 2012). ncRNAs are also implicated in cognitive brain function, e.g., PIWI-interacting RNAs (piRNAs) were recently identified to be abundantly expressed in the CNS and mediate activity induced CpG methylation and transcriptional silencing of key synaptic plasticity-related genes.

The examples discussed above summarize neatly that the epigenetic gene regulation machinery is broadly implicated in neural lineage differentiation, synaptic plasticity, memory formation, and behavior.

7 EPIGENETICS IN IDIOPATHIC GENERALIZED EPILEPSY AND EPILEPTIC ENCEPHALOPATHIES

Epilepsy genetics encompasses genes and loci discovered in association with primary epilepsy syndromes, in which the epilepsy is a primary presenting feature, as well as genes discovered in association with disorders of brain development that are associated with epilepsy (Poduri and Lowenstein, 2011). For many years, only mutations in genes encoding for voltage- or ligand-gated ion channels (e.g., neuronal nicotinic acetylcholine receptor alpha 4, CHRNA4; voltage-gated sodium channel type I, beta subunit, SCN1B; voltage-gated sodium channel type I, alpha subunit, SCN1A; gamma-aminobutyric acid A receptor, beta 2, GABRB2) had been linked to genetic forms of epilepsies, which long led the view that all epilepsies were "channelopathies." However, in the past couple of years novel technologies contributed significantly to gene discovery in monogenic and complex genetic epilepsies

and epileptic encephalopathies, increasing the number of epilepsy-related candidate genes by an order of magnitude to almost one hundred (Helbig and Lowenstein, 2013). Intriguingly, many of these genes identified as probably causative with genetic forms of epilepsy are not ion channels, receptors, or other "typical" epilepsy genes, but comprise, in fact, epigenetic players (Table 1).

Table 1 Epigenetic factors in genetically determined epileptic syndromes

Gene	Epigenetic function	Function in the CNS	Disorder
Generalized/myoclonic epilepsies and epileptic encephalopathies			
ARX	Homeodomain transcription factor regulating KDM5C, affects histone methylation and chromatin remodeling	Maintenance of specific neuronal subtypes in the cerebral cortex, axon guidance	Early infantile epileptic encephalopathy 1 (EIEE1)/West syndrome; X-linked lissencephaly (LISX2); X-linked mental retardation (XLMR)
CHD2	Chromatin remodeler (CHD family)	Not determined	Epileptic encephalopathy, childhood-onset (EEOC)
CDKL5	Dnmt1 phosphorylation, MeCP2-binding protein	Not determined	Early infantile epileptic encephalopathy 2 (EIEE2); atypical Rett syndrome; Angelman
MBD5	Methyl DNA-binding protein	Neurogenesis, cell survival, LTP, memory and learning	syndrome mental retardation, autosomal dominant 1 (MRD1); 2q23.1 microdeletion syndrome with seizures
PRICKLE1	Nuclear receptor, necessary for nuclear localization of REST	Axon guidance	Progressive myoclonic epilepsy 1B (PME1B)
Intellectual disability, autism, and epilepsy			
ATRX	Chromatin remodeler (SWI/SNF family)	Neurogenesis	Alpha-thalassemia/ mental retardation (ATRX)
EHMT1	Histone methyltransferase	Memory and learning	Kleefstra syndrome
FMR1	RNA-binding protein in miRNA pathway	Memory and learning	Fragile X syndrome (FXS)
KDM5C	Histone demethylase	Not determined	X-linked mental retardation (XLMR)

Table 1 Epigenetic factors in genetically determined epileptic syndromes—cont'd

Gene	Epigenetic function	Function in the CNS	Disorder
MECP2	Methyl DNA-binding protein	LTP, memory and learning	Rett syndrome; non-syndromic
NSD1	Histone methyltransferase	Not determined	X-linked mental retardation (XLMR); Angelman syndrome; Autism Sotos syndrome; Beckwith–Wiedemann syndrome
SMARCA4	Chromatin remodeler (SWI/SNF family)	Neurogenesis	Mental retardation, autosomal dominant 16 (MRD16)

ARX, Aristaless-related homeobox; ATRX, alpha thalassemia/mental retardation syndrome X-linked; CHD2, chromodomain 2; CDKL5, cytokine-dependent kinase-like 5; EHMT1, euchromatic histone-lysine methyltransferase 1; FMR1, fragile X mental retardation 1; KDM5C, lysine (K)-specific demethylase 5C; MBD5, methyl-CpG-binding domain 5; MeCP2, methyl-CpG-binding protein 2; NSD1 nuclear receptor-binding SET domain protein 1; PRICKLE1 prickle homolog 1 (Drosophila); SMARCA4: SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 4.

The epigenetic enzymes and effector proteins described to be mutated in inherited genetic epilepsies as well as epileptic encephalopathies, intellectual disability syndromes, and autism spectrum disorders with associated severe or occasional seizure phenotype are of various function. Recent studies identified chromodomain helicase DNA-binding protein 2 (CHD2) as candidate gene in Dravet-like fever associated epileptic encephalopathy (Carvill et al., 2013; Suls et al., 2013). This protein resembles an ATP-dependent helicase with chromatin remodeling function (Marfella and Imbalzano, 2007). Remodelers like the CHD protein family alter gene expression by modification of chromatin structure, meaning that they can help to improve accessibility of the transcriptional apparatus to the DNA template either by moving, ejecting, or restructuring nucleosomes. It is the energy from the hydrolysis of ATP that allows the remodeling complexes to reposition (i.e., slide, twist, or loop) nucleosomes along the DNA, expel histones away from DNA or facilitate exchange of histone variants, and thus create nucleosome-free regions of DNA for gene activation. A different subtype (i.e., SWI/SNF) of chromatin remodelers, including SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2 and 4 (SMARCA2, SMARCA4), and AT-rich interactive domain 1B (ARID1B), has been identified in patients with various mental retardation syndromes and seizures (de la Serna et al., 2006; Ronan et al., 2013; Tsurusaki et al., 2012). Interestingly, loss of SMARCA4 gene expression could be correlated with drug resistance in cancer *in vitro* and *in vivo* (Kobow et al., 2013a). Alpha-thalassemia/mental retardation (ATRX), another chromatin

remodeler of the SWI/SNF family, was found to be mutated in ATRX, which is associated with epilepsy in approximately 30% of patients (Gibbons, 2006; Guerrini et al., 2000; Picketts et al., 1996). 50% of all ATRX syndrome mutations affect a domain required for DNMT3 binding, leading to altered DNA methylation profiles and chromatin structure. The epileptogenic mechanisms may include perturbations of inhibitory interneuron survival and differentiation and, therefore, lead to the deployment of neural networks with an altered balance between excitatory and inhibitory components (Medina et al., 2009; Qureshi and Mehler, 2010).

Histone-modifying enzymes and readers of histone tags are frequently identified in intellectual disability syndromes with associated epilepsy. EHMT1 is a histone methyltransferase that is implicated in learning and memory and was recently shown to be mutated in Kleefstra syndrome (Kleefstra et al., 2006). Among the commonly seen features in these patients are severe mental retardation, epileptic seizures, and behavioral problems. In EHMT1-negative patients with core features of Kleefstra syndrome, but otherwise heterogeneous phenotypes, deleterious *de novo* mutations in four other genes encoding epigenetic regulators were identified including KMT2C, another histone-lysine methyltransferase (Kleefstra et al., 2012). KDM5C is a lysine-specific demethylase, and as such erasing methylation marks from histones. Its function in the CNS is not further determined, but a mutation in this gene has been associated with X-linked intellectual disability (Iwase et al., 2007; Jensen et al., 2005). Different types of mutations of the transcription factor ARX have been linked to Ohtahara syndrome and other epileptic encephalopathies, West syndrome, X-linked myoclonic epilepsy as well as intellectual disability with seizures (Bienvenu et al., 2002; Shoubridge et al., 2010). Intriguingly, ARX is a key regulator of KDM5C function and has therefore an indirect effect on histone methylation patterns (Poeta et al., 2013). Mutations and deletions of the histone methyltransferase nuclear receptor binding SET domain protein 1 (NSD1) 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). Patients with Angelman Syndrome present with large maternal deletions of chromosome 15q11–q13, paternal uniparental disomy (UPD) of chromosome 15, imprinting mutations, or with mutations in the ubiquitin ligase gene UBE3A. Interestingly, Ube3a-deficiency limits dendritic-spine density and maturation. Consequently, new synapse formation is halted resulting in the dysfunction of neuronal networks. Moreover, synaptic function was identified to be perturbed in Ube3a-deficient rat neurons, due to enhanced removal of the AMPA-type glutamate receptors (Scheiffele and Beg, 2010). Clinical features of Angelman syndrome include severe mental retardation with no speech, motor and sensory deficits, and epilepsy.

DNA methylation is essential for genomic integrity, X-chromosome inactivation, and genomic imprinting. It is further critical for a variety of neurobiological and cognitive processes including neurogenesis, stem cell maintenance, synaptic plasticity, as well as learning and memory. MeCP2, a typical reader of DNA methylation, is

most abundant in the brain and, when mutated, the cause of classic Rett syndrome (Amir et al., 1999). Seizures are reported in up to 80% of affected females, with generalized tonic–clonic seizures and partial complex seizures being most common (Jian et al., 2006). MBD5 is another DNA methylation-binding protein and was identified as causal locus of intellectual disability and epilepsy in patients with 2q23.1 microdeletion syndrome (Williams et al., 2010). CDKL5 codes for a cyclin-dependent kinase and mutations in the CDKL5 gene have been associated with atypical Rett syndrome (Bienvenu and Chelly, 2006) as well as X-linked dominant early infantile epileptic encephalopathy (Evans et al., 2005). The protein shows a similar spatiotemporal expression pattern during development as MeCP2. Further, CDKL5 has been proposed capable of interacting with and phosphorylating MeCP2 and DNMT1, thereby influencing gene expression and DNA methylation. 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).

All given examples illustrate the important relationship between epigenetic factors and genetically determined epileptic syndromes. It can be concluded that all epigenetic mechanisms are relevant to maintain brain homeostasis. Aberrations in any of the discussed epigenetic mechanisms can initiate broad changes in gene expression and protein function that may drive neuronal hyperexcitability and seizure formation.

8 EPIGENETICS IN TLE

In a complex disease, knowledge about epigenetic alterations can elucidate the origin of some non-Mendelian inheritance and etiology beyond genetic mutations. In epilepsy, just like in any other complex disease, phenotypic variation and disease susceptibility (e.g., late onset, parent-of-origin effects, discordance of monozygotic twins, and fluctuation of symptoms in dependence of nutrition, hormones, or other environmental aspects) as well as the response to drugs may be compounded by epigenetic anomalies (Huidobro et al., 2013; Kobow et al., 2013a; Petronis, 2001).

TLE is the most common epilepsy syndrome in adults. Seizures originate primarily from the hippocampus, which frequently shows distinct patterns of segmental neuronal cell loss and gliosis (hippocampal sclerosis, HS). Clinical history indicates an early onset of the disease process in some patients, i.e., those exhibiting severe febrile seizures, status epilepticus, or brain inflammation during the first years of childhood. The initial precipitating injury is usually followed by a clinically silent latent period before the onset of epilepsy. Many patients become drug resistant during the course of the disease and possibly need surgical treatment to achieve seizure control. The underlying pathomechanisms have not yet been identified. TLE is not a

genetic disorder in the strict sense, but familial cases of TLE and genetic predisposition to febrile seizures or other risk factors have been described (Berkovic and Scheffer, 2001; Hirose et al., 2003). The majority of cases appear to be associated with acquired focal lesions like HS, tumors, or certain malformations. To gain some insight into the mechanisms underlying TLE, gene expression profiling studies have been performed for over a decade and hundreds of genes were identified to be misregulated in human and experimental TLE, many of which are thought to participate in inflammation and stress, synaptic transmission and signal transduction, ion transport, cell metabolism as well as synaptic plasticity (Becker et al., 2003; Elliott et al., 2003; Gorter et al., 2006; Hendriksen et al., 2001; Lukasiuk et al., 2006). At the same time, a spectrum of chromatin alterations has been identified in different animal models of epilepsy and various stages of epileptogenesis. These profiles include predominantly gene or locus specific and, more recently, also global alterations in epigenetic chromatin modifications, which correlated with changes in gene expression. It has been hypothesized that epigenetic changes may function as master switch of pro-epileptogenic gene expression changes here (Fig. 1; Kobow and Blumcke, 2011, 2012).

Among the candidate genes that have been linked to TLE the ionotropic glutamate receptor AMPA 2 (Gria2, downregulated) and the brain-derived neurotrophic factor (Bdnf, upregulated) along with numerous other genes seem to play a key role in seizure-induced pathological events (Kokaia et al., 1995; Pellegrini-Giampietro et al., 1997; Sanchez et al., 2001; Xu et al., 2004). Intriguingly, both genes harbor response elements for the Nrsf, a central regulator of neuronal gene expression. Nrsf is involved in seizure development and progression and serves to repress gene expression through dynamic recruitment of epigenetic complexes including Dnmts as well as HDACs (Hu et al., 2011; Huang et al., 1999; Liu et al., 2012; Park et al., 2007). Thus, epigenetic regulation of Bdnf and Gria2 gene expression could be assumed. In fact, in a rat model of pilopcarpine-induced status epilepticus, immediate hypo- and hyperacetylation of histones bound to Gria2 and Bdnf promoters were observed following status epilepticus, which corresponded well with a decrease and increase in respective gene expression. Administration of HDAC inhibitor prior to pilocarpine injection reversed these molecular changes (Huang et al., 2002). Histone acetylation appears to be involved in the process of epileptogenesis and TLE irrespective of the model analyzed (Huang et al., 2002; Jia et al., 2006; Tsankova et al., 2004). Seizure-associated Bdnf and Gria2 expression could be also inversely correlated with respective promoter methylation *in vitro* and *in vivo* pointing at DNA methylation-related chromatin remodeling as important mechanism in activity-dependent gene regulation and neural plasticity (Machnes et al., 2013; Martinowich et al., 2003). Histone methylation as well as higher order activation-dependent chromatin remodeling in the neuronal cell nucleus further seem to contribute to the activation of Bdnf and downstream effector genes upon seizures and in memory formation (Gupta et al., 2010; Gupta-Agarwal et al., 2012; Walczak et al., 2013).

Animal models of epilepsy and human tissue studies suggest that epileptogenesis involves a cascade of molecular, cellular, and neuronal network alterations including

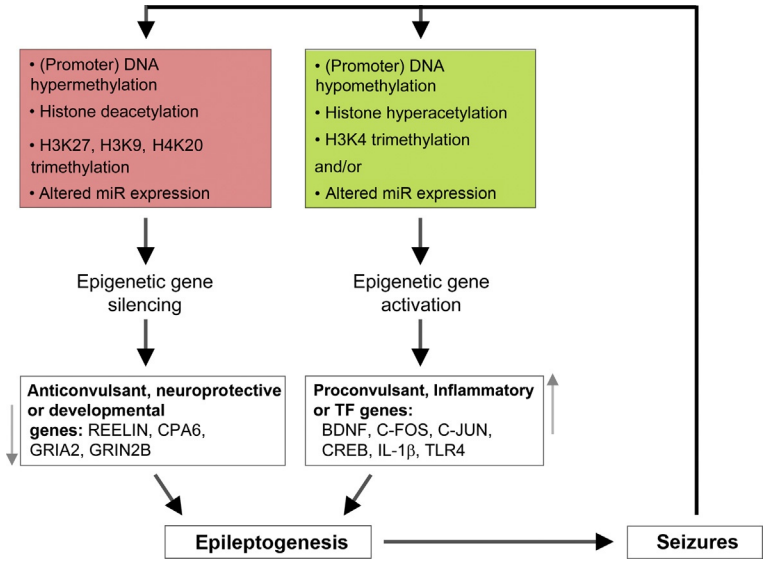

FIGURE 1

Epigenetic mechanisms implicated in epileptogenesis. Epigenetic activation or silencing of genes implicated in epileptogenesis and seizure formation. To current knowledge DNA hypermethylation, loss of histone acetylation together with a combination of inhibitory histone methylation marks or altered miR expression can induce epigenetic gene silencing as could be shown in part for REELIN (Kobow et al., 2009), CPA6 (Belhedi et al., 2014), Gria2 (Huang et al., 2002; Machnes et al., 2013), or Grin2b (Ryley Parrish et al., 2013). In contrast, gene activation could be linked to DNA hypomethylation, increased histone acetylation and H3K4 trimethylation as well as specific miR expression patterns. Genes known to be epigenetically activated in epilepsy and contributing to a pro-epileptogenic state are Bdnf (Martinowich et al., 2003; Ryley Parrish et al., 2013; Walczak et al., 2013), immediate early genes, e.g., c-fos and c-jun (Sng et al., 2005, 2006), as well as the major transcription factor Creb (Qureshi and Mehler, 2010; Sng et al., 2006; Tsankova et al., 2004). Similar mechanisms can be anticipated for inflammatory genes as IL-1β and TLR4 (Maroso et al., 2010; Takahashi et al., 2009). It has been suggested that seizures themselves may induce epigenetic alterations and subsequent gene expression changes, thereby, promoting the pathogenic condition.

activation of immediate early genes (Rakhade and Jensen, 2009). Immediate early or primary response genes frequently comprise viral genes, e.g., c-fos, c-jun, c-myc, encoding for transcription factors, or other DNA-binding proteins. They are characterized by their rapid and transient induction in many cell types in response to a wide range of stimuli and have therefore been considered the "gateway to genomic response." Some IEGs have been implicated in neuronal plasticity, learning and memory, as well as long-term potentiation, proving their significance for higher order brain function under physiologic and pathologic conditions, e.g., epilepsy.

Epigenetic mechanisms have been suggested to regulate IEG expression. Following kainate-induced status epilepticus, c-fos and c-jun promoter-associated histones were found to be regulated by H3 Serine 10 phosphorylation and histone H4 acetylation in rat hippocampal neurons. Changes in histone acetylation and IEG expression spatiotemporally correlated with increased expression of Crebbp, a well-known transcriptional coactivator with intrinsic HAT activity. Pretreatment with curcumin, a HAT inhibitor specific for Crebbp/Ep300, attenuated histone acetylation, decreased IEG expression, and limited the severity of status epilepticus (Sng et al., 2006). Contrarily, treatment with an HDAC inhibitor led to histone hyperacetylation and increased IEG expression after kainic acid administration (Sng et al., 2005). It was further demonstrated in the electroconvulsive-seizure-induced model of epilepsy that the cAMP-responsive element-binding protein 1 (Creb1) gene promoter itself is also subject to selective histone H4 and H3 modifications (Tsankova et al., 2004). Creb1 is an important transcriptional activator regulated through calcium signaling and implicated in modulating a broad array of cellular processes including the differential expression of $GABA_A$ receptor subunits in the epileptogenic hippocampus. This observation suggests that epigenetic modulation of transcription of key neurotransmitter receptors orchestrating the interplay between synaptic excitation and inhibition is involved in mediating epileptogenesis (Qureshi and Mehler, 2010).

Brain inflammation can be frequently observed in epilepsy, but the impact of specific inflammatory mediators on neuronal excitability is not well understood. Inflammatory cytokines including interleukin-1β (IL-1β) are known to be released subsequent to an initial precipitating injury or following recurrent seizures and to promote hyperexcitability, seizure-evoked cell death, or transcriptional activation of NFκB and mitogen-activated protein kinase-dependent genes involved in structural and functional changes of glial and neuronal networks (Vezzani and Baram, 2007). The proconvulsant effects of inflammation can be studied in animals treated with the bacterial endotoxin lipopolysaccharide (LPS). LPS treatment induces distinguished activation of a histone demethylase, thereby blunting H3K9 di- and trimethylation at the IL-1β promoter and activating IL-1β gene expression. This process of LPS-induced epigenetic IL-1β gene activation has been studied in neural stem cells, but possibly could be generally admitted in the mammalian brain (Das et al., 2013). LPS is known to be particularly recognized by the cell surface toll-like receptor 4 (TLR4), which triggers inflammation by inducing the transcription of genes encoding cytokines and as such contributes to the onset and recurrence of seizures. Aberrant high TLR4 expression together with a clear implication in ictogenesis has been described in human and experimental TLE (Maroso et al., 2010). Intriguingly, in other tissue than brain histones within the 5′ region of the TLR4 gene are acetylated in response to LPS and together with promoter methylation regulate TLR4 gene expression (Takahashi et al., 2009).

Evidence for a particular role of DNA methylation in the pathogenesis of seizures came from two *in vitro* studies demonstrating a decrease of spontaneous excitatory neurotransmission and network activity following 5-aza-cytidine (5-aza-C) or

Zebularine-mediated inhibition of Dnmts in both hippocampal slices (Levenson et al., 2006) and hippocampal primary neurons (Nelson et al., 2008). There is further evidence for aberrant DNA methylation of the Reelin (RELN) promoter associated with granule cell dispersion, a frequent migration defect targeting the hippocampal granule cell layer, in TLE patients with HS (Kobow et al., 2009). In TLE patients with HS and known history of febrile seizures most recently increased carboxypeptidase A6 (CPA6) promoter methylation was observed (Belhedi et al., 2014). CPA6 is involved in the selective biosynthesis of neuroendocrine peptides and loss-of-function mutations have been related to seizures and epilepsy (Sapio et al., 2012). Furthermore, increased DNMT1 and DNMT3A expression have been described in temporal neocortex samples obtained from TLE patients, which is in line with the localized DNA methylation changes described above, but could also points to even broader changes of DNA methylation in the pathogenesis of focal epilepsies (Kobow and Blumcke, 2012; Zhu et al., 2011). In fact, methyl-CpG capture-associated massive parallel sequencing (Methyl-Seq) as well as array-based analyses of genomic DNA methylation patterns in two different rodent seizure models identified global changes in DNA methylation following status epilepticus or epileptic tolerance (Miller-Delaney et al., 2012) and identified a methylation signature distinguishing chronic epileptic animals from healthy controls (Kobow et al., 2013b). Localized increase and decrease of DNA methylation could be observed and corresponded well with observed gene expression changes in same animals. In another study, using the kainic acid-induced SE model of TLE global increase in hippocampal DNA methylation was correlated with an increase in Dnmt activity, disruption of adenosine homeostasis, and spontaneous recurrent seizures. Adenosine augmentation over 10 days reversed DNA hypermethylation seen in the epileptic brain, inhibited hippocampal sprouting of mossy fibers, and prevented the progression of epilepsy for at least 3 months (Williams-Karnesky et al., 2013).

These data suggest epigenetic mechanisms to be critically involved in epileptogenesis and propagation of the chronic disease state, accounting for the synergistic misregulation of multiple genes in major pro-epileptogenic pathways including synaptic reorganization, neuroinflammation, or development of pharmacoresistance. In the future, specific epigenetic modification patterns (e.g., DNA methylation) may be of considerable interest as potential biomarker for early detection of disease onset, prognosis, or monitoring of disease after therapy.

9 METABOLISM AND THE EPIGENOME

Neuronal activity accounts for 80% of brain energy consumption. Blood-borne glucose is an essential energy source for the adult human brain. Glucose is taken up by both neurons and astrocytes via their specific glucose transporters (GLUT3 in neurons and GLUT1 in astrocytes) and glucose oxidation occurs via glycolysis. However, the ability of neuronal cells to activate this metabolic pathway is poor particularly in response to synaptic activation. Here, glial cells are key mediators

of neurometabolic coupling, producing lactate from glucose both under basal conditions and upon increased neuronal demand. Lactate is in turn used as oxidative fuel by neurons, which in addition metabolize glucose through the pentose phosphate pathway to keep their redox balance and avert apoptosis. Besides being the brain's primary energetic fuel, glucose is also a remarkably versatile precursor supplying metabolic intermediaries for biosynthetic reactions. In brain, most of these intermediaries serve to synthesize neurotransmitters, as well as other molecules of biological significance. Under certain conditions, the brain can utilize acetoacetate, β-hydroxybutyrate, and acetone (ketone bodies) derived from fatty acids as alternative energy source (Prins, 2008). Neurons metabolize ketone bodies to acetyl-coenzyme A (acetyl-CoA), which is further oxidized through the tricarboxylic acid cycle (TCA cycle). High-circulating levels of ketone bodies are known to protect the brain tissue against intractable epilepsy. Ketone bodies also prevent seizures in GLUT1 deficiency syndrome (Klepper, 2008) and are important for brain development (Prins, 2008). The cellular and molecular mechanisms underlying the protective effect of ketone bodies are not clear and various hypothesis have been put forward (Masino and Rho, 2012). Here, we will discuss a possible link of metabolism with epigenetic changes in the normal and epileptic brain (Fig. 2).

Epigenetic marks are initiated, perpetuated, and removed via the activity of numerous enzymes (e.g., DNMTs, HATs, HDACs, HMTs among many others). They continually modify and constitute chromatin to alter its structure and refine downstream function, i.e., gene expression. Histone- and DNA-modifying enzymes perceive metabolic changes by requiring cofactors from numerous biochemical pathways to power their effort, which constitutes a connection between gene expression and metabolism (Hitchler and Domann, 2012). HATs catalyze the acetylation of lysine residues in the N-terminal tails of core histone proteins using acetyl-CoA as cofactor thereby linking glycolysis, fatty acid, and amino acid metabolism with one key epigenetic mechanism (Fig. 2). Antagonizing histone acetylation and repressing gene expression is mediated by HDACs, of which the Sirtuin family of proteins uses nicotinamide adenine dinucleotide (NAD^+) as cofactor to break the bond between lysine and acetyl group (Fig. 2). The requirement for NAD^+ by Sirtuins vividly exemplifies how metabolism through epigenetic mechanisms can be a driver of gene expression patterns (Hitchler and Domann, 2012). This has been discussed in the context of cancer (Hitchler and Domann, 2012), but may also be relevant to epilepsy. Seizure activity can lead to energy failure just like mutations affecting metabolic genes implicated in the maintenance of cellular energy homeostasis often result in an epileptic phenotype. Both findings imply that energy failure can contribute to epileptogenesis with evidence particularly for the contribution of TCA cycle deficits in generating seizures (Kovac et al., 2013).

Numerous studies have demonstrated that histone acetylation is altered in epilepsy and may correlate with disease progression (Huang et al., 2002; Sng et al., 2005, 2006). HAT and HDAC activity is dependent upon the availability of cofactors (i.e., acetyl-CoA, NAD^+) and a stunning association exists between protein acetylation and glycolysis. When glycolysis exceeds the cell's aerobic metabolic capacity

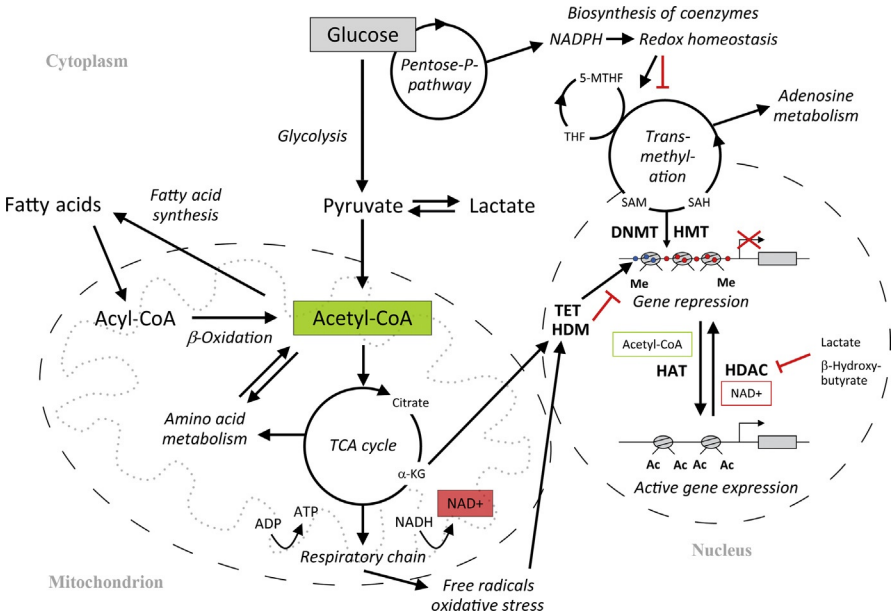

FIGURE 2

Metabolism affects epigenetic gene regulation via availability of cofactors. Scheme of metabolic processes (*italic*) in brain cells and their interaction with epigenetic mechanisms, e.g., histone acetylation and DNA methylation, in the nucleus. Upon increased neuronal activity and metabolic demand, glial cells take up blood-borne glucose, which is metabolized in the cytoplasm through glycolytic oxidation to generate pyruvate. When glycolysis exceeds the cell's aerobic metabolic capacity lactate accumulates and can enter the nucleus, where it acts as endogenous HDAC inhibitor. Pyruvate is converted to acetyl-coenzyme A and supplied to the TCA cycle which fuels the respiratory chain and is intensively linked to fatty and amino acid metabolism. Concentrations of acetyl-CoA and NAD^+ affect histone acetylation and thereby couple cellular metabolic status and transcriptional regulation. Interestingly, defects in many TCA cycle enzymes can lead to epilepsy in humans. Neurons easily utilize lactate released from glia, covert it back to pyruvate, and use it in oxidative metabolism. Nevertheless, neurons need to continuously utilize glucose in the pentose phosphate pathway to keep their redox homeostasis and prevent apoptosis. Changes in the cellular redox buffering capacity directly influence enzymes involved in the transmethylation pathway affecting key epigenetic mechanisms, i.e., DNA and histone methylation, and gene expression. There is a strong interplay of the transmethylation pathway with folate and adenosine metabolism. Mutations in enzymes of the transmethylation pathway, folate cycle as well as shifts in adenosine metabolism have been linked with epilepsy in humans. Ac, acetyl group; HAT/HDAC, histone acetyltransferase/deacetylase; Me, methyl group; HMT/HDM, histone methyltransferase/demethylase; red (dark gray in the print version) dots, DNA methylation, 5-mC; blue (dark gray in the print version) dots, DNA hydroxymethylation, 5-hmC; DNMT, DNA methyltransferase; TET, ten-eleven translocation (DNA-demethylating enzyme); ADP/ATP, adenosine di-/triphosphate; $NAD^+/NADH_2$, nicotinamide adenine dinucleotide (hydroxide); 5-MTHF/THF, (5-methyl) tetrahydrofolate; SAM/SAH, *S*-adenosylmethionine/-homocysteine.

lactate accumulates and can enter the nucleus, where it acts as endogenous HDAC inhibitor (Fig. 2). Although weaker than other HDAC inhibitors including trichostatin A and butyrate, lactate induces hyperacetylation and increased gene expression in a broad range of target genes all commonly affected by HDAC inhibitor treatment (Latham et al., 2012). β-Hydroxybutyrate, which is closely related to butyrate, is the major source of energy for mammals during prolonged exercise or starvation and has selective inhibitory function for class I HDACs (Shimazu et al., 2013). Inhibition of HDACs by β-hydroxybutyrate might contribute to the beneficial effect of the ketogenic diet in epilepsy treatment. Another ketone body, important TCA cycle intermediate and anaplerotic carbon source, α-ketoglutarate, is an essential cofactor of Jumonji C domain-containing histone demethylases (Cascella and Mirica, 2012). TET protein family members, which are involved in active DNA *de*methylation via oxidation of 5-mC to 5-hmC, are also activated by α-ketoglutarate (Loenarz and Schofield, 2008). Isocitrate dehydrogenases 1 and 2 (IDH1/2) are $NADP^+$-dependent enzymes of the TCA cycle that normally catalyze the conversion of isocitrate to form α-ketoglutarate. As cofactor of TET1, α-ketoglutarate levels are directly associated with global DNA hydroxymethylation (Chia et al., 2011). Under oxidative stress, an increase in the ratio of NAD^+ to NADH is observed affecting Sirtuin 3, a NAD^+-dependent protein deacetylase, which activates IDH2 and favors synthesis of 2-hydroxyglutarate over α-ketoglutarate. Subsequent allosteric inhibition of histone and DNA demethylation by 2-hydroxyglutarate further leads to altered gene expression (Chia et al., 2011). Experimental evidence clearly supports an involvement of oxidative stress in seizure generation (Patel, 2004) and via α-ketoglutarate availability seems to affect epigenetics and gene activity in the epileptic brain (Hitchler and Domann, 2012).

Neurons metabolize glucose through the pentose phosphate pathway to keep their redox balance, even under conditions when lactate is used as primary oxidative fuel. Altered cellular redox buffering capacity affects enzymatic activity of the methionine adenosyltransferase (MAT), which uses the methyl group provided by 5′-methyl tetrahydrofolate (5′-MTHF) and the amino acid methionine to regenerate SAM as methyl group donor for DNA methylation. A more reducing environment increases MAT activity and SAM production. Conversely, a more oxidized environment has been proposed to decrease MAT activity and SAM levels (Hitchler and Domann, 2012). Disturbances in folate metabolism (e.g., low-folate diet or MTHFR mutations/reduced enzymatic activity) have been associated with increased CSF levels in HCY, a proconvulsant, and seizures (Baldelli et al., 2010; Chen et al., 2001; Goyette et al., 1995; Ono et al., 2000). Alterations in adenosine metabolism have also been associated with epilepsy in rodents and humans (Boison, 2008). A constant shift toward hydrolysis of SAH resulting from efficient adenosine removal has been suggested to effectively promote transmethylation (Gouder et al., 2004).

The experimental data summarized here provide evidence that disruption in the production and availability of metabolic intermediates like SAM, α-ketoglutarate, β-hydroxybutyrate, lactate, NAD^+, and acetyl-CoA can modify the epigenotype of neuronal and glial cells. Redox biology can also change epigenetic events through

oxidation of enzymes and alterations of metabolic cofactors that affect epigenetic events like DNA methylation. Combined, these metabolic and redox changes could serve as the molecular basis for altering the epigenotype of normal cells and may help create the epigenetic progenitor of any pathological condition including epilepsy (Hitchler and Domann, 2012).

10 BALANCING THE EPIGENOME: THERAPEUTIC STRATEGIES

Unlike genetic changes, epigenetic modifications are reversible, which suggests that DNMTs, HATs and HDACs, and HKTs and HDMTs may be promising therapeutic targets (Kelly et al., 2010). Genome-wide screens of histone modifications and DNA methylation have provided an unbiased means to define diagnostic epigenetic signatures for cancers and from the initial findings in epilepsy research it can be hypothesized that specific signatures can also be identified in the epileptic brain, most probably even cell-specific patterns.

A well-established treatment option in medically refractory epilepsies is the high-fat, low-carbohydrate ketogenic diet. The ketogenic diet has been proposed to act through glycolytic inhibition. As described above, small metabolic intermediates (NAD^+, acetyl-CoA, α-ketoglutarate, β-hydroxybutyrate) are thought to act as regulators of epigenetic enzymes including REST (Garriga-Canut et al., 2006), HDACs of the Sirtuin family, Jumonji C domain-containing histone demethylases, and TET enzymes involved in active DNA demethylation, thereby linking energy availability to chromatin structure and transcriptional output.

Beyond endogenous epigenetically active cofactors, there is a growing number of drugs designed or reinvented that selectively target certain classes of epigenetic enzymes. Drugs that inhibit DNMT activity are mainly nucleoside analogues including 5-aza-C (Vidaza, FDA approved), 5-aza-2′-deoxycytidine (5-aza-dC; Decitabine, FDA approved), and Zebularine. Both 5-aza-C and 5-aza-dC serve as suicide substrates to DNMTs targeting these enzymes for proteasomal degradation and reducing global DNMT levels primarily in rapidly proliferating cells (Eglen and Reisine, 2011). To avoid typical toxicity and stability problems associated with nucleoside inhibitors, non-nucleoside DNMT inhibitors have been developed. RG108 is a small epigenetic compound and specific inhibitor of DNMT1 that displays antiproliferative, but not cytotoxic, properties (Brueckner et al., 2005). Intriguingly, DNMT expression and activity are elevated in human and rodent epilepsy (Williams-Karnesky et al., 2013; Zhu et al., 2011). A number of repositioned drugs approved for other indications such as the antihypertensive drug hydralazine, or the local anesthetic procaine, or even the antiarrhythmic drug procainamide have also been identified as non-nucleoside DNMT inhibitors all either targeting DNMTs directly or inhibiting signaling pathways associated with DNA methylation (Kobow and Blumcke, 2012). Another novel approach addresses the physiological RNA interference pathway to silence DNMTs. *MG98* is an antisense oligonucleotide specifically binding to the 3′ untranslated region (3′UTR) of DNMT1 mRNA and targeting

mRNA degradation. A clinical phase I trial in tumor patients has proved its safety and tolerability as well as early evidence of antitumor activity (Plummer et al., 2009). Physiologic micro-RNAs targeting *DNMT* gene expression may also represent interesting targets for pharmacological intervention, e.g., miR-29 and miR-143, but have not yet been addressed in pre-/clinical trials (Kobow and Blumcke, 2012).

HDACs, which are classified into five main subtypes: classes I, IIa and IIb, and IV, and the structurally distinct class III (Sirtuins), have been recognized as useful therapeutic targets for a broad range of human disorders including cancer and a growing number of neurologic diseases (e.g., Autism, Huntington's, and motor neuron disease) (Hahnen et al., 2008). Many HDACs show both nuclear and cytosolic localization and interact with numerous (also non-histone) protein partners to execute multiple functions. Small-molecule HDAC inhibitors have been shown to restore transcriptional balance to neurons, modulate cytoskeletal function, affect immune responses, and enhance protein degradation pathways. Class IIa HDACs (HDAC4, HDAC5, HDAC7, and HDAC9) seem to be an interesting target due to their selective expression also in the brain, suggesting a specific role for these proteins in CNS function (Kazantsev and Thompson, 2008). Sirtuins, the class III HDACs, recently have been implicated in neurogenesis, metabolism, and aging (Saharan et al., 2013). They are structurally very distinct from other HDAC subtypes and therefore can be selectively targeted. The first compound bearing an HDAC inhibitory function, sodium butyrate, was identified in the late 1970s. Valproic acid (VPA), the most commonly used antiepileptic drug, has been FDA approved in 1987, but only in 2001 it has been discovered to possess HDAC inhibitory function. VPA and sodium butyrate show pronounced activity against class I HDACs at medium to high millimolar doses; thus, providing very low efficacy compared to second generation HDAC inhibitors suberoylanilide hydroxamic acid (Vorinostat, Zolinza, FDA approved in 2006), Romidepsin (FDA approved in 2009), LBH598, and MS-275 among many others (Gottlicher et al., 2001).

Histone demethylases classify into LSD family members and Jumonji C domain-containing enzymes and are considered putative drug targets, because several members have a role in cancers or neurologic disease. Mutations in KDM5C have been associated with X-linked mental retardation, whereas haploinsufficiency of KDM6A was found in patients with severe psychomotor retardation, global growth restriction, and seizures. Despite a considerable pharmacological interest, no truly promising drug candidates that selectively target histone demethylases have been published so far. The catalytic domains of the LSD proteins share sequence homology with monoamine oxidases, which play an important role in the metabolism of neuroactive amines in the CNS including Dopamine and Serotonin and can be targeted by tranylcypromine (FDA approved to treat psychological disorders). Inhibitors of JMJC demethylases have been reported, but few have sufficient potency and selectivity. Most of the reported inhibitors are metal chelators that bind competitively with the 2-oxoglutarate cofactor (Hojfeldt et al., 2013).

Combined these data show that a growing number of small epigenetically active compounds, e.g., targeting DNA methylation and histone modifications are currently

under development, with the primary aim to optimize the specificity of target interaction as well as route of administration to reduce unfavorable side effects.

11 SUMMARY

Alterations in cell signaling by environmental changes can remodel epigenetic marks, which thought to serve as mechanism for transcriptional "plasticity" that mediates sustained variation in neural function. Epigenetic signatures contribute to the molecular memory of any given cell and may explain sustained changes in transcriptional activity during cell differentiation, learning and memory, chronic stress, environmental toxins, and pathogenic conditions including seizures and epilepsy (Das and Chai, 2013). Studying epigenetic chromatin modifications opens fascinating new avenues for our understanding of common pathomechanism of epileptogenesis, and novel epigenetically active pharmacological compounds may be recognized as antiepileptic treatment. Currently, global effects of epigenetic inhibitors impede normal cellular functions, which is a clear limitation and severe side effects must be envisaged for unselective systemic epigenetic drug treatment (Hatzimichael and Crook, 2013; Harden et al., 2009). Therefore, future studies need to address how the enzymology may be specifically targeted to the affected brain region or even to selected epilepsy-associated genes.

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