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Epigenetic Mechanisms in Stroke and Epilepsy

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Epigenetic remodeling and modifications of chromatin structure by DNA methylation and histone modifications represent central mechanisms for the regulation of neuronal gene expression during brain development, higher-order processing, and memory formation. Emerging evidence implicates epigenetic modifications not only in normal brain function, but also in neuropsychiatric disorders. This review focuses on recent findings that disruption of chromatin modifications have a major role in the neurodegeneration associated with ischemic stroke and epilepsy. Although these disorders differ in their underlying causes and pathophysiology, they share a common feature, in that each disorder activates the gene silencing transcription factor REST (repressor element 1 silencing transcription factor), which orchestrates epigenetic remodeling of a subset of 'transcriptionally responsive targets' implicated in neuronal death. Although ischemic insults activate REST in selectively vulnerable neurons in the hippocampal CA1, seizures activate REST in CA3 neurons destined to die. Profiling the array of genes that are epigenetically dysregulated in response to neuronal insults is likely to advance our understanding of the mechanisms underlying the pathophysiology of these disorders and may lead to the identification of novel therapeutic strategies for the amelioration of these serious human conditions.

INTRODUCTION

Epigenetics is influenced by environmental cues and has a role in all aspects of neuronal function, from embryogenesis and early brain development to tissue-specific gene expression and global gene silencing; therefore, it is reasonable to believe that dysregulation of epigenetics would have a significant role in neuropsychiatric disorders such as ischemic stroke and epilepsy (Levenson and Sweatt, 2005; Graff et al, 2011). Gene transcription is regulated by methylation of DNA at CpG dinucleotides and covalent modifications of highly conserved amino-acid residues in core histone proteins, which in turn modifies chromatin structure and the accessibility of chromatin to the core transcriptional machinery (Wood et al, 2006; Berger, 2007; Robison and Nestler, 2011). Chromatin-modifying proteins catalyze post-translational modifications, such as acetylation, methylation, ubiquitination, phosphorylation, SUMOylation, and adenosine diphosphate (ADP) ribosylation, which serve as epigenetic tags that promote or repress gene transcription (for review, see (Jenuwein and Allis, 2001; Kouzarides, 2007; Berger, 2007; Brandl

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et al, 2009)). Moreover, emerging evidence points to a widespread role for microRNAs as modulators of translation of target genes at early stages of brain development, and are critical to dendritogenesis, synapse formation and synapse maturation (Schratt, 2009a; Siegel et al, 2011b). The epigenome is large and complex and the modifications can work together in various combinations to create different biological responses. This diverse and dynamic repertoire provides a mechanism by which genetic predisposition, experience, and changes in neural function can operate together to create cellular and behavioral responses. Dysregulation of the vast epigenome could contribute to the complex cause for neurological disease and disorders.

This article reviews the role of epigenetic modifications of genes involved in synaptic function and plasticity and how dysregulation of these genes contribute to pathological conditions with cognitive impairments. In addition, the article highlights the potential power of drugs that target the epigenetic machinery in developing new therapeutic approaches for two neurological disorders, stroke and epilepsy. These two disorders are of particular interest because unlike late-onset, progressive neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease, stroke and epilepsy can arise from a single traumatic event. Moreover, there is considerable comorbidity in these two disorders; 11% of adult onset epilepsy occurs as a result of a

stroke (Rakhade and Jensen, 2009), indicating that there may be common triggers and mechanisms underlying impaired memory for these two disorders.

EPIGENETIC MODIFICATONS

Epigenetic modifications such as DNA methylation and histone modifications reflect environmental influences that are not 'hard-wired' into the DNA sequence and are critical to genomic reprogramming during normal brain development, tissue-specific gene expression and global gene silencing (Levenson and Sweatt, 2005; Graff *et al*, 2011). During normal brain development, the connections between neurons and neuronal circuitry undergo remodeling in response to environmental cues. An emerging view is that environmental factors can also contribute to disruptions in synaptic signaling and neuronal survival (Day and Sweatt, 2011). Thus, epigenetic modifications such as methylation of DNA and histones are associated not only with learning and memory, but also with deficits in memory arising as a consequence of ischemic stroke or seizures.

DNA Methylation

DNA methylation is a key epigenetic signaling tool used by the cell to lock genes in the 'off' position. Properly established and maintained patterns of DNA methylation are essential for normal brain development, lineage commitment, and normal functioning of the adult organism (Robertson, 2005; Guibert *et al*, 2009; Senner, 2011). DNA methylation provides stable indexing marks that extend over long chromosomal domains, giving rise to 'memorized' states of gene expression that may be inherited from one cell generation to the next (Borrelli *et al*, 2008; Day and Sweatt, 2011). Methylation of mammalian DNA is catalyzed by DNA methyltransferases (DNMTs), chromatin-modifying proteins that establish and maintain unique DNA methylation patterns in different cells in different contexts. Three active members of the DNMT family (DNMT1, DNMT3a, and DNMT3b) are expressed in mammals. Although DNMT1 ensures faithful maintenance of DNA methylation, DNMT3a and DNMT3b are essential for *de novo* DNA methylation (Okano *et al*, 1999; Song *et al*, 2012). While DNA methylation at a gene promoter is typically associated with transcriptional repression, DNA methylation in gene-bodies is typically associated with active gene transcription (Ball *et al*, 2009; Laurent *et al*, 2010).

Aberrant DNA methylation is implicated in a wide range of human diseases, including cancer, imprinting disease, repeat-instability neurobehavioral diseases such as Fragile X syndrome (Robertson, 2005), neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and disorders such as ischemic stroke and epilepsy (Petrij *et al*, 1995; Bartsch *et al*, 2005; Roelfsema *et al*, 2005; Urdinguio *et al*, 2009; Qureshi and Mehler, 2010a).

Histone Modifications and Transcriptional Regulation

Nucleosomes are the basic unit of chromatin and are composed of 147 bp DNA wrapped around a histone octamer (two each of H2A, H2B, H3, H4), linker histone (H1) and histone variants (H2A.X, H2A.Z, H3.3) (Ruthenburg *et al*, 2007). Each histone has a central domain and unstructured N-terminal tails that contain multiple potential sites for post-translational modifications including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP ribosylation. These marks are added or removed by a specific set of chromatin-modifying enzymes (Ruthenburg *et al*, 2007). Distinct, site-specific histone modifications act sequentially or in combination to form a 'histone code' read by other proteins to bring about distinct downstream events, which in turn mediate active gene transcription or repression (Ruthenburg *et al*, 2007).

Histone Acetyltransferases

Histone acetyltransferases (HATs) confer acetyl moieties to lysine and arginine residues in the N-terminal tails of core histone proteins. Acetylation relaxes chromatin structure, enabling the transcriptional machinery to access the transcriptional start site. HATs not only modify histones, but also other substrates such as transcription factors (Kouzarides, 2000), and are therefore also referred to as lysine acetyltransferases to reflect their array of targets (Aka *et al*, 2011). Although HATs can affect global gene expression by acetylating large regions of chromatin, including coding and non-promoter regions, they can also act in a gene-specific manner by acetylating core histones at specific gene promoters (Dekker and Haisma, 2009; Selvi *et al*, 2010).

Histone acetylation has a critical role in memory and synaptic function and its dysregulation is linked to neurological disease. For example, mutations in the genes encoding either of the two well-known HATs, CBP and p300, leads to Rubinstein–Taybi syndrome, an autism spectrum disorder (ASD) characterized by intellectual disabilities and physical abnormalities (Petrij *et al*, 1995; Bartsch *et al*, 2005; Roelfsema *et al*, 2005; Urdinguio *et al*, 2009). In addition, CBP has a role in the pathophysiology of Huntington disease (Jiang *et al*, 2006).

Histone Deacetylases

The control of histone acetylation depends on the balance between two sets of chromatin-modifying enzymes, HATs and histone deacetylases (HDACs). The HDAC superfamily is made up of four classes (class I, II, III, IV), with class I and II being the most studied with prominent expression in the brain (for review see: (Gray and Ekstrom, 2001; de Ruijter *et al*, 2003; Morrison *et al*, 2007; Haggarty and Tsai, 2011)). HDACs remove acetyl groups from core histone proteins, thereby condensing the chromatin and repressing

transcription. HDACs also remove acetyl moieties from other proteins such as transcription factors and thereby alter cellular function (Morrison *et al*, 2007; Morris *et al*, 2010). HDACs do not bind DNA directly, but rather function as part of a multi-protein complex to recognize other factors such as transcriptional activators and repressors, which direct them to their target genes (de Ruijter *et al*, 2003; Carey and La Thangue, 2006).

Histone Methylation

Methylation occurs not only on cytosine bases in DNA, but also on lysine and arginine residues in the N-terminal tails of histone proteins (Scharf and Imhof, 2011). The impact of marks of methylation is complex in that its impact is determined in a site-specific manner and by the number of methylation marks that are conferred at a specific residue. The impact of methylation is site-specific, in that it can promote either gene activation or gene repression, depending on the exact lysine or arginine that is marked. Activation is signaled by methylation of lysines 4, 36, and 79 on histone 3 (H3K4, H3K36, H3K79) and repression is signaled by methylation of lysines 9 and 27 on histone 3 and lysine 20 on histone 4 (H3K9, H3K27, H4K20) (Kouzarides, 2007; Borrelli *et al*, 2008). Methylation marks are reversible in that they are removed by histone demethylases such as lysine-specific demethylase 1 (LSD1) (Lee *et al*, 2005; Shi, 2005). Rather than changing chromatin structure directly, the main function of methylation is to attract proteins that can modify chromatin (Cloos, 2011). Methylation itself is also important in cognitive functions and intellectual disabilities (Tahiliani *et al*, 2007). Moreover, histone demethylases are implicated in diseases such as cancer, autism, X-linked mental retardation, and schizophrenia (Cloos, 2011).

MicroRNAs in Epigenetic Remodeling

microRNAs are short, single-stranded RNAs that regulate gene expression post-transcriptionally, typically by binding recognition motifs within the $3'$-untranslated regulatory regions (UTRs) of target mRNAs (Siegel *et al*, 2011b). Although best known for their role in regulation of translation (Kasinski and Slack, 2011; Salta and De, 2012), microRNAs can also regulate RNA transcription (Kim *et al*, 2008; Benhamed *et al*, 2012). Importantly, microRNAs regulate expression of key epigenetic regulators including DNMTs, HDACs, polycomb group proteins, and MeCP2 (Sato *et al*, 2011). Moreover, a subset of microRNAs such as let-7a, miR-9, miR-34a, miR-124, miR-137, miR-148, and miR-203 are regulated by epigenetic mechanisms including DNA methylation and histone modification (Sato *et al*, 2011). Interestingly, although miR-9 represses REST (repressor element 1 silencing transcription factor) and miR-9* represses CoREST, REST itself represses miR-9 and miR-9* (Packer *et al*, 2008), Thus, microRNAs can participate in 'epigenetic-miRNA regulatory circuits' (Sato *et al*, 2011).

Emerging evidence points to a widespread role for microRNAs as key modulators of target gene expression in neurons. microRNAs have a pivotal role in the late stages of neural differentiation, maintenance, and plasticity (Schratt, 2009b) and early stages of brain development including dendritogenesis, synaptogenesis, and synapse maturation. A key feature of neuronal microRNAs is their ability to regulate networks of neuronal or nonneuronal genes in an activity- or experience-dependent manner (Schratt, 2009b). Moreover, microRNAs achieve spatial specificity of gene expression in that they can regulate mRNA translation locally in the synaptodendritic compartment in an activity-dependent manner (Schratt, 2009b). As an example, miR-132 is critical for hippocampal spine formation and the inflammatory response (Shaked *et al*, 2009; Magill *et al*, 2010). miR-132 is thought to function together with FMRP to regulate translation in dendrites. Dysregulation of miR-132 and its target genes is implicated in the pathophysiology of Fragile X syndrome (Edbauer *et al*, 2010) and schizophrenia (Miller *et al*, 2012).

STROKE

Stroke is the leading cause of disabilities in adults in the United States, and is the second leading cause of death worldwide (Moskowitz *et al*, 2010; Ofengeim *et al*, 2011). Of the 600 000 new victims each year, 30% die and another nearly 30% become severely and permanently disabled. Most phase III clinical trials conducted to date have failed. Currently, there is no known treatment or cure for the neurodegeneration associated with this debilitating and devastating disease. It is thus critical that new molecular mechanistic information be revealed and be brought into the translational arena with the goal of identifying new therapeutic strategies for neuroprotection, rescue, and repair.

Transient forebrain or global ischemia arises as a consequence of cardiac arrest, cardiac surgery, profuse bleeding, near-drowning, and carbon monoxide poisoning and affects $\sim 200\,000$ Americans each year (Liou *et al*, 2003; Moskowitz *et al*, 2010; Ofengeim *et al*, 2011). Neurological sequelae include deficits in learning and memory. Although all forebrain areas experience oxygen and glucose deprivation, a brief ischemic insult causes cell death primarily of CA1 pyramidal neurons, critical to memory formation and retrieval. Neuronal death, exhibiting characteristics of apoptosis and necrosis, is not detected until 2–3 days after ischemia (Liou *et al*, 2003; Moskowitz *et al*, 2010; Ofengeim *et al*, 2011). By 1 week after ischemia, the CA1 pyramidal cell layer can be virtually ablated. During the ischemic episode, cells exhibit a transient, early rise in intracellular Ca^{2+}, depolarize and become inexcitable; ambient glutamate rises by $\sim$4-fold to $\sim 2\,\mu M$. After reperfusion, cells appear morphologically normal, exhibit normal intracellular Ca^{2+} and regain the ability to generate action potentials for 24–72 h after the insult. A myriad of death cascades are activated, including caspase-dependent and independent apoptosis, mitochondrial release of cytochrome *c*,

Smac/DIABLO and AIF, excitotoxicity, lipid peroxidation, and swelling (Liou et al, 2003; Lo et al, 2003; Zukin et al, 2004). Ultimately, there is a late rise in intracellular Ca^{2+} and Zn^{2+}, and death of CA1 neurons ensues, exhibiting hallmarks of apoptosis and necrosis. Although the molecular mechanisms underlying ischemia-induced death are not definitively understood, the substantial delay between insult and neuronal death is consistent with a role for transcriptional changes in cell death (Liou et al, 2003; Moskowitz et al, 2010; Ofengeim et al, 2011).

Transcriptional Regulation by REST During Stroke

REST, also known as NRSF (neuron-specific silencing factor), is a gene-silencing transcription factor that is widely expressed during embryogenesis and has a strategic role in terminal neuronal differentiation (Roopra et al, 2001; Ballas and Mandel, 2005; Ooi and Wood, 2007). In pluripotent stem cells and neural progenitors, REST actively represses a large array of coding and noncoding neuron-specific genes important to synaptic plasticity and structural remodeling, including synaptic vesicle proteins, channels, neurotransmitter receptors, and microRNAs that regulate networks of neuronal or non-neuronal genes. Bioinformatics analysis predicts nearly 2000 putative REST targets in the mammalian genome, including both coding and noncoding genes (Bruce, 2004; Conaco et al, 2006; Qureshi and Mehler, 2009). The interplay between REST and microRNAs is a new, emerging topic (Conaco et al, 2006; Singh et al, 2008; Johnson et al, 2008; Packer et al, 2008). During the late stages of neuronal differentiation, loss of REST is critical to acquisition of the neuronal phenotype (Su et al, 2006).

In mature neurons REST is quiescent, but can be activated in selectively vulnerable hippocampal neurons by insults such as global ischemia (Calderone et al, 2003; Formisano et al, 2007; Noh et al, 2012) and epileptic seizures (Palm et al, 1998; Garriga-Canut et al, 2006; Gillies et al, 2009) and aberrantly accumulates in the nucleus of selectively vulnerable striatal neurons in Huntington's disease (Zuccato et al, 2003, 2007).

A fundamental mechanism by which REST silences target genes is that of epigenetic remodeling (Borrelli et al, 2008). REST binds the RE1 element of target genes and recruits CoREST (Andres et al, 1999; Ballas et al, 2001) and mSin3A (Naruse et al, 1999; Huang et al, 1999; Grimes et al, 2000; Roopra et al, 2001), corepressor platforms which in turn recruit HDACs-1 and 2. HDACs deacetylate core histone proteins and affect dynamic and reversible gene silencing (Roopra et al, 2001; Ballas and Mandel, 2005; Ooi and Wood, 2007). REST mediates long-term gene silencing by associating with the site-specific histone methyltransferase G9a, which promotes dimethylation of histone 3 at lysine 9 (H3K9me2) via CoREST-dependent (Lunyak et al, 2002; Ballas et al, 2005) and -independent (Roopra et al, 2004) mechanisms and with LSD1, which removes mono-and di-methyl groups from lysine 4 of H3 (Lee et al, 2005; Shi,

2005). In addition, REST recruits methyl-CpG-binding protein 2 (MeCP2) (Lunyak et al, 2002; Ballas et al, 2005), a transcriptional repressor, which binds to hotspots of methylated CpG dinucleotides in gene promoters where it complexes with other repressors (Feng and Nestler, 2010). Importantly, MeCP2 is a reader of epigenetic marks on histones and DNA (Borrelli et al, 2008).

A prevailing view has been that REST is a master transcriptional regulator of neuronal genes in pluripotent stem cells and neural progenitors and that loss of REST during the late stages of neural differentiation by ubiquitin-based proteasomal degradation (Westbrook et al, 2008; Guardavaccaro et al, 2008) is required for acquisition of the neural phenotype (Ballas et al, 2005). An earlier paper by our group broadened this view (Calderone et al, 2003). Experiments involving molecular and genetic approaches showed that ischemic insults trigger activation of REST in mature hippocampal neurons destined to die and that the increase in REST correlates with a decrease in histone acetylation and gene silencing of GluA2. This is significant in that the GluA2 subunit prevents Ca^{2+} influx via AMPA receptors (AMPARs), is essential to synapatogenesis, long-lasting forms of synaptic plasticity and neuronal death (Kwak and Weiss, 2006; Liu and Zukin, 2007). Acute knockdown of REST in hippocampal slices subjected to oxygen glucose deprivation (OGD), an in vitro model of ischemia, prevented GluA2 downregulation and neuronal death (Calderone et al, 2003). While compelling, these findings raised new questions. Are other synaptic proteins regulated by REST in insulted neurons? Are REST and corepressors recruited to the promoters of target genes and, if so, does the corepressor complex orchestrate epigenetic remodeling and gene silencing? Is REST causally related to neuronal death in a clinically relevant model of ischemic stroke?

A more recent study by our group employed a targeted ChIP–chip analysis with a microarray containing all known functional targets of REST to identify genes with altered REST occupancy in CA1 tissue from animals subjected to global ischemia (Noh et al, 2012). Of note, REST was enriched at promoters of a subset of target genes vital to synaptic function, of which gria2, the gene that encodes the AMPAR subunit GluA2, was the most affected. These findings represent an advance over previous studies, in which they show that, in addition to gria2, other genes important to synaptic plasticity and structural remodeling are functional targets of REST in fully differentiated neurons destined to die. Moreover, these results reinforce the notion that, although REST can repress thousands of putative target genes, the subset that is transcriptionally responsive is cell-type- and context-specific.

To address the mechanism by which REST silences target genes, a more in-depth analysis of gria2 was performed. The gria2 gene was the leading candidate in the ChIP-chip analysis (Noh et al, 2012). Moreover, the role of GluA2 silencing and expression of GluA2-lacking, Ca^{2+}-permeable AMPARs in neuronal death are well-established (Kwak and Weiss, 2006; Liu and Zukin, 2007; Isaac et al, 2007).

A critical factor in ischemic cell death is GluA2-lacking AMPARs, which mediate influx of toxic Ca^{2+} and Zn^{2+} in vulnerable CA1 neurons (Pellegrini-Giampietro et al, 1992; Gorter et al, 1997; Opitz et al, 2000; Calderone et al, 2003). The GluA2 subunit renders AMPARs impermeable to Ca^{2+}/Zn^{2+}, modifies channel kinetics and conductance and receptor targeting to synapses (Liu and Zukin, 2007). Thus, an alteration in gria2 gene expression would have profound implications for synaptic plasticity and neuronal survival (Liu and Cull-Candy, 2000; Liu et al, 2004; Liu and Zukin, 2007). GluA2 is a known gene target of REST (Myers et al, 1998; Huang et al, 1999).

Ischemia induced enrichment of REST, mSin3A, CoREST, G9a, and MeCP2, at the gria2 promoter in postischemic CA1 (but not CA3). CoREST and mSin3A serve as corepressor platforms that recruit HDACs 1 and 2, key components of the REST complex, which promote removal of acetyl groups from core histone proteins H3 and H4. Ischemia induced site-specific deacetylation of H3, indicative of gene repression. REST also recruits G9a (Ooi and Wood, 2007; Qureshi and Mehler, 2009), a protein that promotes site-specific methylation of H3. Ischemia induced methylation of H3 at lysine 9, but not lysine 4, an epigenetic signature of gene repression. These findings indicate that the REST complex is active in insulted CA1 and document epigenetic remodeling of the REST target GluA2 in neurons destined to die (Noh et al, 2012) (Figure 1a).

The affinity of REST on gria2 could be due to the fact that it is well known in the chromatin world that the epigenetic landscape at promoter sites determines the binding affinity of DNA-binding proteins/regulatory proteins, which act to repress or promote active gene transcription. The enrichment or depletion of DNA-binding proteins at a given promoter site depends not only on the cellular or nuclear abundance, but also on binding affinity of the regulatory element for the binding protein (Bruce et al, 2009; Yu et al, 2011). An additional and nontrivial point to account for the increased REST affinity is that of multivalency, the situation in which two or more DNA-binding proteins or epigenetic marks bound at the same promoter act synergistically to promote repression (or activation) and alter the affinity/capacity for a given DNA-binding protein, relative to the affinity observed in the absence of the marks (Ruthenburg et al, 2007).

To address a causal relation between REST-dependent epigenetic remodeling and neuronal death, the impact of the HDAC inhibitor trichostatin A (TSA) was examined (Wang et al, 2012; Noh et al, 2012). A single, acute injection of the HDAC inhibitor TSA, administered to animals before or after an ischemic episode, ameliorated neuronal injury. This finding documents a role for epigenetic remodeling in the neuronal death associated with ischemic stroke. Moreover, it has important clinical implications and suggests that HDAC inhibitors may be a promising avenue for intervention in the neurodegeneration associated with ischemic stroke. To address a role for REST in ischemia-induced neuronal death, two additional strategies were undertaken (Noh et al, 2012). We showed that RNAi-mediated knockdown or inhibition of REST by dominant-negative REST prevented epigenetic remodeling, GluA2 silencing and neuronal death. These findings indicate that REST is causally related to epigenetic remodeling and neuronal death and adds ischemic stroke to the growing list of diseases involving REST dysregulation (Figure 1a). Moreover, the study has broad implications for our understanding of molecular mechanisms underlying neurodegenerative disorders.
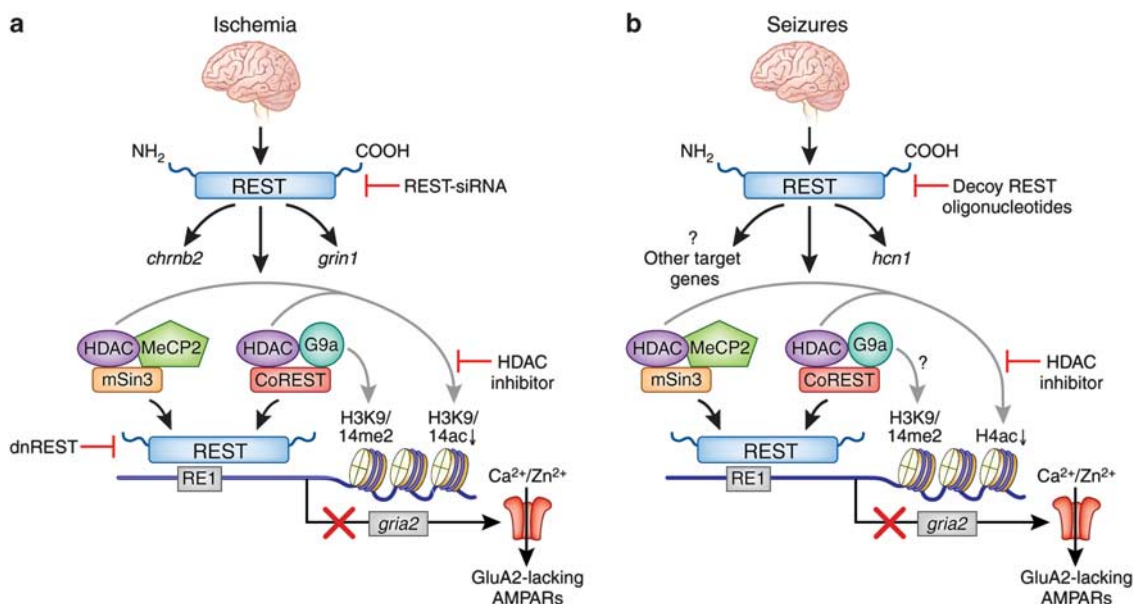

Figure 1. Model showing REST-dependent epigenetic remodeling of the gria2 promoter in response to ischemic stroke or seizures. Global ischemia (left) or seizures (right) activate REST. REST binds to the RE1 element within the promoter of its target gene gria2 and recruits mSin3A and CoREST, HDACs-1/2, G9a and MeCP2. The REST-corepressor complex promotes epigenetic remodeling of core histone proteins at the gria2 promoter. This, in turn, represses GluA2 expression, leading to formation of GluA2-lacking, Ca^{2+}-permeable AMPARs. Modified with permission from (Noh et al, 2012).

DNA Methylation in Animal Models of Stroke

Several studies have explored global alterations in DNA methylation associated with animal models of ischemic stroke. In one study, induction of focal ischemia by means of transient MCAO markedly increased DNA methylation and neuronal death in the cerebral cortex of wild-type mice. Administration of 5-aza-2′-deoxycytinine, a demethylase and broad-spectrum inhibitor of DNMT, conferred neuro-protection in wild-type mice (Endres et al, 2000). Moreover, mice heterozygous for the gene encoding DNMT1 ($Dnmt^{S/+}$) exhibited reduced methylation and increased resistance to ischemic damage, consistent with the concept that enhanced DNA methylation, and presumably gene silencing, are associated with enhanced susceptibility to brain injury (Endres et al, 2000). Although transgenic mice with reduced levels of DNMT1 were resistant to cerebral ischemia-induced brain injury, mice with complete deletion of DNMT1 did not show protection (Endres et al, 2000). These findings suggest that the levels of DNMT expression and DNA methylation must be tightly regulated to protect neurons from ischemic insults.

In recent years, new technologies have enabled identifica-tion and quantitative assessment of alterations in DNA methylation at specific loci in the genome in response to ischemic insults. A study that links ischemia to alterations in DNA methylation at promoters of specific genes focused on the Na^+-K^+-$2Cl^-$ (NKCC1) co-transporter type-1 gene (Lee et al, 2010). It is well established that NKCC1 is important in GABA(A) receptor-mediated responses that switch from depolarization to hyperpolarization during postnatal development. NKCC1 is downregulated during postnatal development (Rivera et al, 1999), but can be reactivated in adult neurons in response to focal ischemic injury (Pond et al, 2006). Consistent with this, DNA methylation of the NKCC1 promoter increased during postnatal development, but decreased in response to ischemic injury (Lee et al, 2010). These findings are consistent with the notion that DNA methylation of the NKCC1 gene promoter is responsible for the increase in NKCC1 expression in postischemic neurons.

Histone Modifications and Transcriptional Regulation in Stroke

In the past decade HDAC inhibitors have emerged as potential therapeutics for stroke. A number of studies have shown the ability of HDAC inhibitors to afford neuroprotection in the MCAO model of ischemic stroke (Gibson and Murphy, 2010). In one study, Chiarugi and colleagues reported that the potent broad-spectrum HDAC inhibitor suberoylanilide hy-droxamic acid (SAHA) administered intraperitoneally to mice at times after induction of MCAO prevented deacetylation of core histone H3, promoted expression of prosurvival proteins Bcl-2 and Hsp70 and reduced infarct volume, indicating a neuroprotective action for SAHA (Faraco et al, 2006).

Several additional studies indicate that the broad spectrum HDAC inhibitor TSA confers protection against ischemia-induced neuronal death. One study showed that TSA promotes neuronal viability following OGD in vitro and reduces neuronal injury in wild-type mice subjected to MCAO in vivo (Wang et al, 2012). Neuroprotection by TSA in these models occurred through activation of the transcription factor, nuclear factor erythoroid 2 (Nrf2) and its downstream targets (Wang et al, 2012). As an example, Nrf2 binds the promoter of the antioxidant sulfiredoxin and increases its expression, which promotes neuronal survival in the face of oxidative stress (Soriano et al, 2009). The second study showed that a single, acute injection of TSA administered to animals after global ischemia in vivo, a clinically relevant model of ischemic stroke, ameliorates neuronal injury (Noh et al, 2012), further emphasizing the role of histone acetylation in ischemia-induced neuronal death. A more recent study demonstrated that not only SAHA, but also the class 1 HDAC inhibitor MS-275, afford protection against OGD-induced neuronal death, promote functional recovery of axons, and preserve white matter cellular architecture (Baltan et al, 2011b). Moreover, HDAC inhibitors conserved ATP levels and reduced excitotoxicity, thereby preserving white matter structure and function (Baltan et al, 2011b).

Histone methylation and demethylation also affect the pathology of global ischemia. As an example, transient, forebrain global ischemia upregulates the site-specific deme-thylase LSD1, which removes methyl groups from lysine 4 on core histone protein H3 (H3K4) (Zhang et al, 2010). The upregulation occurs in hippocampal CA1 and dentate gyrus (DG), as well as in cerebral cortex. Of these regions, highest levels of LSD1 were observed in the DG, a region relatively resistant to neuronal death. Although the increase in LSD1 expression was rapid in the hippocam-pal CA1 and DG (<1 h), it was induced later (by 6 h) in cortex. These differences could contribute to differences in neuronal viability observed in the hippocampus vs cortex after ischemia.

Another study that revealed a role for histone methylation in the neuronal death associated with global ischemia, focused on the gene encoding μ-opioid receptor (MOR-1). MOR-1 is a member of the G protein-coupled receptor superfamily and is activated by endogenous opioid peptides (Kieffer and Gaveriaux-Ruff, 2002). In rats subjected to global ischemia, MOR-1 is downregulated in the hippocampal CA1 (Formisano et al, 2007). Ischemia promoted REST binding to the MOR-1 promoter by 12 h after ischemia, assessed by ChIP, consistent with the possibility that REST mediates MOR-1 downregulation. Moreover, ischemia promoted dimethylation of lysine 9 (H3K9), but not lysine 4 (H3K4) on histone 3 at the MOR-1 promoter, an epigenetic signature of gene repression. MOR-1 is expressed on GABAergic inhibitory interneurons of the CA1, where it inhibits the release of GABA onto pyramidal cells and thereby increases neuronal excitability. Thus, downregulation of MOR-1 in selectively vulnerable CA1 neurons after ischemia is neuro-protective, and may represent a failed attempt to promote neuronal survival (Formisano et al, 2007).

The Role of microRNAs in Stroke

Recent studies indicate striking alterations in microRNA expression in response to ischemic stroke and ischemic preconditioning in animal models and in young patients who suffer stroke (for review see (Rink and Khanna, 2011)). Neuronal death resulting from focal ischemia induced experimentally in animals occurs via caspase-dependent and -independent mechanisms (Du et al, 2004; Yuan et al, 2009; Liu et al, 2009; Siegel et al, 2010). The X-linked inhibitor of apoptosis (XIAP) is an endogenous inhibitor of caspases that has a pivotal role in the response to ischemic insults. Under physiological conditions, XIAP expression is higher in the brains of female vs male mice and is significantly decreased in response to stroke in females, but not males (Siegel et al, 2011a). A recent study provides evidence that miR-23a is a key regulator of XIAP expression. Ischemia promotes an increase in miR-23a expression and its enrichment at the 3'-UTR of XIAP in the cortex of female, but not male, mice. These findings implicate a possible role for miR-23a in the severity and functional outcome of stroke in a gender-specific manner (Siegel et al, 2011a).

Heat-shock proteins (HSP) are a class of functionally related proteins involved in protein folding and unfolding and cell survival. Dramatic upregulation of HSPs in response to stress is known as 'the heat-shock response'. The HSPs of the 70 kDa family, HSP72 (cytosol), GRP75 (mitochondria), and GRP78/BIP (endoplasmic reticulum), are evolutionarily conserved proteins that promote neuronal survival/protection in animal models of stroke (Rajdev et al, 2000; Hoehn et al, 2001; Kudo et al, 2008; Oida et al, 2008). A recent study identified miR-181 as a possible regulator of several HSP70 family members and validated HSP GRP78/BIP to be a target of miR-181 in response to ischemic brain injury. In this study, miR-181 increased in the core, where cells die, but decreased in the penumbra, where cells survive. The increase in miR-181a was associated with a decrease in GRP78, implicating translational repression. Although overexpression of miR-181a exacerbated neuronal death, reduced miR-181a activity increased GRP78 protein expression and reduced neuronal death (Ouyang et al, 2012). These data implicate miR-181 expression in repression of GRP78 and induction of neuronal death.

EPILEPSY

Epilepsy, defined as recurrent unprovoked seizures, affects 1–2% of the population world-wide (Hauser et al, 1993) and ranks second worldwide in prevalence among all neurological disorders (Rakhade and Jensen, 2009). Cognitive impairment, particularly memory disruption, is a major complicating feature of epilepsy. Sensory, motor, or autonomic function can also be affected (Fisher et al, 2005; Berg et al, 2010). Current therapies are largely unsatisfactory, as they suppress seizures, but do not affect the underlying cause, are effective in only a subset of affected individuals, and are often toxic (Duncan et al, 2006; Galanopoulou et al, 2012). Epilepsy refers to a constellation of disorders and can be classified according to characteristic symptoms and signs, seizure type, cause, age of onset, and electroencephalographic patterns (Berg et al, 2010). For many years it was thought that epilepsy occurred exclusively as a result of cortical damage (McNamara, 1999). It is now known that sporadic epilepsy can also arise as a consequence of traumatic brain injury, stroke, abnormalities in brain wiring, toxic-metabolic etiologies, inflammation, autoimmunity, or an imbalance in the ratio of inhibitory to excitatory synaptic transmission (Berg et al, 2010). Epilepsy can also arise as a consequence of spontaneous or inherited gene mutations. Interestingly, the latter includes a vast number of channelopathies, in which recurrent seizures arise from single-point mutations, deletions, duplications, or expansions in genes encoding a component of a voltage- or ligand-gated ion channels, but also many other genes that directly or indirectly control brain development and neuronal function and activity (Catterall et al, 2008, 2010; Galanopoulou et al, 2012).

Epileptogenesis is the process whereby a neural network acquires the ability to manifest recurrent epileptic seizures de novo and in response to neuronal insults, as well as the development and progression of epilepsy (Rakhade and Jensen, 2009; Galanopoulou et al, 2012). Epileptogenesis has been conceptualized as a 'cascade' of molecular and cellular events (Rakhade and Jensen, 2009). In the first phase of epilepsies owing to structural/metabolic etiologies, seizures may develop on the order of minutes to days in response to a neuronal insult such as traumatic brain injury, and are accompanied by acute changes to neuronal networks and activation of immediate early genes (Rakhade and Jensen, 2009). Next, a silent or latent-stage ensues, involving alterations in transcription, neuronal death, and re-wiring, or inflammation. In addition, changes in neurogenesis, sprouting, and reorganization of neuronal networks contribute to the development of spontaneous seizures that increase in frequency over time and can last for weeks to months or years (Rakhade and Jensen, 2009). Emerging evidence indicates that alterations not only in transcription, but also epigenetic mechanisms, including DNA methylation, histone code modifications, chromatin remodeling, and modulation of the epigenetic machinery by noncoding RNAs are involved in the pathogenesis of human epilepsy and in the process of epileptogenesis (Qureshi and Mehler, 2010b).

Comorbidity with Autism

Children with epilepsy often have intellectual and developmental disabilities and co-morbidity of epilepsy with disorders such as anxiety, attention-deficit hyperactivity disorder and/or autism (Russ et al, 2012). A prominent co-morbidity is that of children diagnosed with both autism and epilepsy (Berg et al, 2010; Tuchman et al, 2010; Bolton

et al, 2011). Approximately 20–30% of children with autism have seizures, with the highest rate in autistic children with severe intellectual disabilities (Amiet et al, 2008; Berg et al, 2010; Tuchman et al, 2010). Moreover, six percent of children who suffer a seizure very early in life (aged 1–2 years) develop ASDs with intellectual disabilities (Clarke et al, 2005). Although the mechanisms responsible for this high co-morbidity are as yet unknown, it is postulated that dysregulation of epigenetic programming may be involved.

As an example, Rett syndrome is an ASD, a neurobehavioral disorder characterized by mental retardation, stereotyped behaviors and recurrent seizures (Jian et al, 2006). Approximately 80% of children diagnosed with Rett syndrome exhibit seizures. Rett syndrome typically arises as a consequence of loss-of-function mutations in the X-linked gene MeCP2 (Amir et al, 1999; Abrahams and Geschwind, 2008). This finding links seizures not only to ASDs, but also to deficits in the epigenetic machinery. However, the correlation between onset and severity of epilepsy and MeCP2 genotype in Rett syndrome patients is controversial. Although some studies report such a correlation (Jian et al, 2006; Nectoux et al, 2008), others do not (Nissenkorn et al, 2010).

Other examples of ASDs that present with seizures are Prader–Willi syndrome and Angelman syndrome, ASDs that present with intellectual disabilities and developmental delays (Abrahams and Geschwind, 2008). Given that DNA methylation is critical for genomic imprinting, and that the defective gene in both syndromes lies within imprinted domains at 15q11-q13, it is likely that dysregulation of DNA methylation has a role. Although Prader–Willi syndrome arises from loss of paternally expressed genes in this region, Angelman syndrome arises from the loss of a single gene, ube3a, the gene encoding maternally expressed ubiquitin protein ligase E3A, which is imprinted only in the brain (Abrahams and Geschwind, 2008). Ube3a is required for maintaining plasticity during experience-dependent neocortical development, consistent with the notion that loss of neocortical plasticity contributes to the cognitive deficits associated with Angelman syndrome (Yashiro et al, 2009).

Transcriptional Regulation by REST in Epilepsy

The original paper that linked seizures to epigenetic remodeling of synaptic proteins was pioneered by Timmusk and colleagues who showed that kainate-induced seizures in rats, an animal model of status epilepticus, induces REST mRNA expression rapidly (<3 h after onset of seizures) in the hippocampal CA3 (Palm et al, 1998). A follow-up study by Dingledine and colleagues using a related animal model of status epilepticus, showed that seizures promote deacetylation of core histone protein H4 (a mark of gene repression) at the RE1 site of the gria2 promoter (gene encoding the AMPAR subunit GluA2), while promoting an increase in acetylation of H4 (a mark of open chromatin and active gene transcription) at the promoter of brain-derived neurotrophic factor BDNF (Huang et al, 2002; Tsankova

et al, 2004). Although GluA2 expression was decreased, leading to an increase in GluA2-lacking, Ca^{2+}-permeable AMPARs at CA3 synapses and neuronal death in CA3 (Grooms et al, 2000; Sanchez et al, 2001; Huang et al, 2002), BDNF expression was increased (Kokaia et al, 1995; Binder et al, 1999; Huang et al, 2002; Tsankova et al, 2004). Alterations in expression of these proteins contribute to the pathophysiology of recurrent seizures. Administration of the HDAC inhibitor TSA to rats before induction of seizures prevented and rapidly reversed the increase in histone deacetylation at the gria2 promoter and blunted the downregulation of GluA2 (Huang et al, 2002). These findings link epigenetic remodeling to seizure-induced silencing of GluA2 and, indirectly, seizure-induced neuronal death. Because gria2 is a known target of REST, these findings also implicate REST-dependent epigenetic remodeling of GluA2 in response to seizures (Huang et al, 1999) (Figure 1b).

Another REST target implicated in epilepsy is the hyperpolarization-activated cyclic adenosine monophosphate-gated channel type 1 (HCN1). In normal brain, the HCN1 channel mediates the I(h) current, and keeps in check the intrinsic excitability of pyramidal cell dendrites in the entorhinal cortex and hippocampus (Lewis and Chetkovich, 2011). Accordingly, HCN1 null mice exhibit increased dendritic excitability and susceptibility to seizures in an experimental model of temporal lobe epilepsy (Huang et al, 2009). A recent study showed that seizures promote an increase in REST expression, enrichment of REST at the hcn1 promoter, dimethylation of lysine 9 on core histone protein H3 (H3K9), an epigenetic mark of gene repression, and silencing of the hcn1 gene (McClelland et al, 2011). Thus, REST-dependent epigenetic remodeling represses HCN1 expression and I(h) currents, leading to increased dendritic excitability and epileptiform activity. Disruption of REST binding to the hcn1 promoter by means of decoy oligodeoxynucleotides prevented hcn1 repression and restored its ability to maintain normal levels of dendritic excitability. Collectively, these studies indicate that REST is causally related to HCN1 repression, decreased dendritic excitability, and enhanced epileptiform activity in entorhinal cortical layer III pyramidal neurons (Shah et al, 2004; Jung et al, 2007, 2011) (Figure 1b).

DNA Methylation in Epilepsy

Recent studies provide evidence that aberrant DNA methylation may contribute to the pathophysiology of epilepsy. In the adult brain, the extracellular matrix protein Reelin is important for normal synaptic plasticity, dendritic morphology, and cognitive function (Herz and Chen, 2006). In the developing brain, Reelin helps maintain the correct laminar structure of granule cells in the DG. Brain specimens from humans diagnosed with temporal lobe epilepsy commonly show dispersion of granule cells, an anatomical hallmark of epilepsy, and a deficiency in expression of Reelin (Haas et al, 2002; Heinrich et al, 2006). A recent study revealed hypermethylation at the reelin promoter (Kobow et al, 2009), suggesting that

aberrant DNA methylation is causally related to the low levels of Reelin in the DG and link aberrant methylation to the pathophysiology of human epilepsy.

Another recent study performed an unbiased, genome-wide study of altered DNA methylation in tissue samples from the CA3 of control, epileptic tolerant and status epilepticus mice (Miller-Delaney et al, 2012). Epileptic tolerance is an evolutionarily conserved form of cerebral plasticity in which a brief period of seizures ('epileptic preconditioning') confers transient tolerance to a subsequent bout of seizures in the brain. A total of 321 genes showed altered DNA methylation in animals subjected to status epilepticus or to epileptic preconditioning (Miller-Delaney et al, 2012). Of these, 293 genes (>90%) showed a decrease in DNA methylation (hypomethylation). Only 15 genes, many of which were associated with nuclear function such as DNA binding and transcriptional regulation, were hypermethylated. These results were surprising, given that epileptic tolerance elicits transcriptional silencing it was thought that DNA would exhibit hypermethylation. Importantly, the genome-wide methylation profiles revealed upregulation of novel genes, not previously associated with epilepsy, such as the polycomb group proteins, in the vulnerable CA3 of epileptic tolerant animals. Polycomb proteins act via epigenetic mechanisms to silence potential mediators of neuronal death and promote cellular arrest, enabling adult neurons to survive neuronal insults (Zukin, 2010).

Histone Modification and Transcriptional Regulation in Epilepsy

Emerging evidence indicates that aberrant histone modifications and altered gene expression are hallmark features of animal models of status epilepticus and humans with temporal lobe epilepsy. As an example, a recent study showed that status epilepticus in rats promotes hyperacetylation of H4 and phosphorylation of H3 (marks of active transcription) at the c-fos, c-jun, and cbp promoters and increases c-fos, c-jun, and CBP expression (Sng et al, 2006). c-fos and c-jun are immediate early genes that are activated rapidly and transiently in response to cellular stimuli and promote cell growth, differentiation, and neuronal survival. CBP is a HAT and essential co-activator of the prosurvival transcription factor CREB. Pretreatment of rats with curcumin, an active component of turmeric with antioxidant, anti-inflammatory, and neuroprotective properties, and a HAT inhibitor, attenuated histone modifications, expression of immediate early genes c-fos and c-jun, and severity of status epilepticus (Sng et al, 2006). Together, these data suggest that histone modifications such as acetylation and phosphorylation have a pivotal role in regulation of genes involved in neuronal survival.

Another recent study showed that HDAC2, an HDAC implicated in brain development (Guan et al, 2009; Morris et al, 2010), is upregulated in humans with temporal lobe epilepsy and in animal models of status epilepticus (Huang et al, 2012). This is significant in that HDAC2 is a negative

TABLE 1 microRNAs with Altered Expression in Epilepsy

Upregulated microRNAs in epilepsy		
Jimenez-Mateos et al (2011)	Hu et al (2011)	Song et al (2011)
miR-10b	miR-22	miR-9*
miR-21	**miR-24**	miR-23a
miR-27a	miR-26a	miR-23b
miR-29a	miR-29b	**miR-24**
miR-30e	miR-30c	miR-27b
<u>miR-132</u>	miR-34a	**miR-29a**
miR-134	**miR-99a**	**miR-99a**
miR-139	miR-101-1	miR-126
miR-146b	miR-124a	miR-127
miR-153	miR-125a	<u>miR-132</u>
miR-199	miR-125b	**miR-134**
miR-200a	<u>**miR-132**</u>	miR-137
miR-219	miR-145	miR-139-5p
miR-323	miR-150	miR-140*
miR-326	miR-151	miR-146a
miR-375	miR-196b	miR-324-5p
miR-425	miR-199a	miR-379
miR-451	miR-213	miR-383
miR-487b	**miR-375**	
miR-507		
miR-509		
miR-518d		

Downregulated microRNAs in epilepsy		
Jimenez-Mateos et al (2011)	Hu et al (2011)	Song et al (2011)
miR-133b	miR-10b	miR-98
miR-330	miR-21	miR-185
miR-374	miR-25	miR-352
miR-381	miR-29a	let-7E
miR-422b	miR-181b	let-7D
miR-497	miR-181c	
miR-657	miR-215	

regulator of cognitive function (Graff et al, 2012). Accordingly, HDAC2 represses genes associated with synaptic plasticity and memory formation (erg1, creb1, bdnf, NMDA receptor subunits, grin1, grin2a, grin2b, and c-fos). These findings implicate upregulation of HDAC2 in the cognitive impairments associated with temporal lobe epilepsy.

Methylation of lysine residues in the N-terminal histone tail is dynamically regulated by the opposing actions of histone methyltransferases such as G9a and histone demethylases such as LSD1 and KDM5C (SMCX). KDM5C removes di- and tri-methyl marks from lysine 4 of H3 and represses gene transcription. Mutations in the histone demethylase KDM5C are linked to X-linked mental retardation and epilepsy (Tahiliani et al, 2007). KDM5C effects epigenetic remodeling and gene silencing by assembling with the transcriptional repressor REST and its corepressors

(CoREST, HDAC1 and -2, and G9a). Notably, KDM5C promotes REST-dependent repression of a subset of target genes such as BDNF and sodium channel type 2A (SCN2A) (Tahiliani et al, 2007). These genes are of interest because increases in their activity are implicated in the pathophysiology of epilepsy (Binder et al, 2001; Shi et al, 2011). Inactivating mutations in KDM5C impairs REST-dependent silencing of neuronal genes such as BDNF and SCN2A, thereby intensifying seizures and contributing to X-linked mental retardation.

In addition, heat-shock 27-kDa-associated protein 1 (HSPBAP1), a member of the histone demethylase family JmjC, is expressed in the anterior temporal neocortex of patients with intractable epilepsy, but not in normal controls (Xi et al, 2007; Cloos, 2011). Although it is not yet known by which mechanism upregulation of HSPBAP1 contributes to epilepsy or neuronal death, an attractive scenario is that HSPBAP1 represses expression of the protective protein HSP27, thereby exacerbating neuronal death.

The Role of microRNAs in Epilepsy

Recently, the ability of status epilepticus to alter the microRNA expression profile in the selectively vulnerable hippocampal CA3 was evaluated by means of microRNA array analysis, an unbiased, epigenome-wide approach to detect alterations in microRNA expression (Jimenez-Mateos et al, 2011; Hu et al, 2011; Song et al, 2011). In Table 1, we summarize alterations in microRNA expression associated with status epilepticus from three independent studies. In all three studies, miR-132 was strikingly upregulated in the hippocampal CA3 after status epilepticus (Table 1, underlined). miR-24, miR-29a, miR-99a, miR-134, and miR-357 were increased in at least two of the three studies (Table 1, boldface), identifying these microRNAs as regulated in response to seizures. In contrast, the three studies showed no consensus (or overlap) in downregulated microRNAs (Table 1, Lower panel). GO analysis classified upregulated microRNAs as components of ERK-MAPK signaling, TGF-β signaling, and/or tight junctions (Hu et al, 2011). A subset of the downregulated microRNAs are implicated in synaptic plasticity, and T-cell receptor signaling.

Alterations in expression of many of the microRNAs identified in the array analysis (miR-132, miR-let-7, miR-23a/b, miR-34a, miR-22, miR-125a, miR-21) were individually validated. For example, miR-132 was shown to be increased in the CA3 in an animal model of status epilepticus. In vivo microinjection of antagomirs against miR-132 depleted miR-132 in CA3. A functional role of miR-132 in anti-inflammation has been identified (Shaked et al, 2009). Since inflammation and blood–brain barrier opening are implicated in epileptogenesis, it is thought that increased miR-132 may contribute to epileptogenesis (Vezzani et al, 2011). In addition, miR-146, another inflammation-associated microRNA, is increased in reactive astrocytes (Aronica et al, 2010) These studies document involvement of microRNAs in regulation not only in neurons, but also in astroglia.

Preliminary studies in animal models of stroke and epilepsy indicate that specific microRNAs may serve as potential biomarkers for these neurological disorders (Liu et al, 2010). A recent study investigated microRNA expression profiles in the hippocampus and blood of animals 24 h after they were subjected to global ischemia or kainic acid-induced status epilepticus. Analysis of their results identified specific microRNAs that changed at least two-fold in response to these neuronal insults. Following ischemia, miR-182 was upregulated and miR-223 and miR-210 were downregulated in both blood and brain; in addition, four microRNAs (miR-29c, miR-34b-3p, miR-98, miR-122) were downregulated in both blood and brain after epilepsy (Liu et al, 2010). These data support the possibility that a subset of microRNAs may prove useful as biomarkers in that blood levels of these microRNAs correlate with their levels in the injured hippocampus.

NOVEL THERAPEUTIC STRATEGIES

In the past decade, an explosion of studies has linked epigenetic modifications to human diseases such as ischemic stroke and epilepsy. Spurred with this information, the pharmaceutical industry has promoted a number of drugs that target the epigenetic machinery as potential therapeutic strategies to ameliorate the symptoms associated with stroke and epilepsy. An important focus has been on drugs that can penetrate the brain–blood barrier and have minimal toxicity. Several such drugs have been developed and are approved by the US Food and Drug Administration (FDA). The most common drugs in clinical trials to date are DNMT inhibitors and HDAC inhibitors (Table 2).

DNMT Inhibitors

The use of DNMT inhibitors as a pharmacological strategy is a quickly growing field. As an example, mice subjected to MCAO exhibit aberrant DNA methylation; administration of the DNMT inhibitor 5-aza-2′-deoxycytidine affords neuroprotection (Endres et al, 2000). Although DNMT inhibitors have not, as yet, been tested in animal models of status epilepticus or in humans with epilepsy, the finding of aberrant DNA methylation in status epilepticus (Kobow et al, 2009; Miller-Delaney et al, 2012) suggests that pharmacological agents that reverse DNA methylation are a high priority. The first generation of DNMT inhibitors developed for humans (Table 2) block DNMT function by preventing unbinding of DNMT from DNA. This, in turn, prevents methylation at neighboring sites on the DNA, leading to global demethylation, which would account, at least in part, for off-target effects (Kelly et al, 2010). Use of DNMT inhibitors for amelioration of neurological disorders may be difficult for a number of reasons. First, stable and bioavailable compounds would need to penetrate the blood–brain barrier to reach the affected brain region. These drugs would need to be of high potency and specificity to minimize off-target effects. Second, as these drugs are known to work well

TABLE 2 Drugs that Target the Epigenetic Machinery

	DNMT inhibitors/HDAC inhibitors	Developmental stage	Specificity	Basic research in epilepsy or stroke
DNMT inhibitors	5-Azacytidin (azacytidine, Vidaza)	Approved 2004	DNMTs	
	5-Aza-2-deoxycytidin (Dacogen, decitabine)	Approved 2006	DNMTs	Endres et al (2000, 2001)
	MG98	Phase I/II	DNMT1	
	Zebularine	Preclinical	DNMTs	
HDAC inhibitors	Valproic acid (VPA)	Approved 1986	Class I and II	Wang et al (2011); Xuan et al (2012)
	SAHA (Zolinza, vorinostat)	Approved 2006	Class I and II	Faraco et al (2006); Baltan et al (2011a, b)
	Romidepsin (Depsipeptid)	Approved 2009	Class I	
	LBH589 (Panobinostat)	Phase III	Class I and II	
	MS-275 (Entinostat)	Phase II	Class I	
	MGCD0103 (Mocetinostat)	Phase II	Class I	
	CI-994	Phase II	Class I and II	
	PXD101 (Belinostat)	Phase II	Class I and II	
	Baceca	Phase II	Class I	
	Savicol	Phase II	NA	
	PCI-24781 (Abexinostat)	Phase II	Class I and II	
	ITF2357 (Givinostat)	Phase II	NA	
	Kevertin	Phase I	Class I	
	CUDI-101	Phase I	Class I and II	
	AR-42	Phase I	Class I and II	
	4SC-202	Phase I	Class I	
	ACY-1215	Phase I	Class II	
	Sulforaphane	Phase I	Class I and II	

in rapidly dividing cells, they may be less efficacious in treating postmitotic cells such as neurons. Third, DNMT inhibitors would need to be continually administrated over days or weeks (Kelly et al, 2010). Recent studies have therefore focused on development of second-generation DNMT inhibitors, which have increased specificity and reduced toxicity. An example is MG98, a second-generation antisense oligonucleotide, which inhibits translation of the mRNA encoding human DNMT1 (Plummer et al, 2009). This and related second-generation DNMT inhibitors hold promise for the amelioration of the neuronal death and cognitive deficits associated with stroke and epilepsy.

HDAC Inhibitors

HDAC inhibitors are actively and intensively being evaluated in pre-clinical studies and clinical trials for their ability to intervene in the neurodegeneration and cognitive deficits associated with stroke and epilepsy. In pre-clinical studies, four main classes of HDAC are under investigation: the short-chain fatty acids, such as sodium butyrate, phenylbu-tyrate, and valproic acid, the hyroxamic acids, such as TSA and SAHA, the epoxyketones, such as trapoxin, and the benzamides (Abel and Zukin, 2008). SAHA and TSA promote neuronal survival in animal models of ischemia (Gibson and Murphy, 2010; Baltan et al, 2011b; Wang et al, 2012; Noh et al, 2012) and epilepsy (Huang et al, 2002; Kobow and Blumcke, 2011). Intriguingly, valproic acid, which is commonly prescribed as an anticonvulsant drug,

was subsequently discovered to be an HDAC inhibitor (Gottlicher et al, 2001). In spite of their neuroprotective actions in epilepsy and stroke, valproic acid and other broad-spectrum HDAC inhibitors can cause global DNA demethylation (Dong et al, 2010) and histone acetylation (Eleuteri et al, 2009), resulting in cell death. These findings suggest that HDAC inhibitors are relevant and feasible targets for developing clinical drugs, but at the same time, point to the need to develop new HDAC inhibitors with greatly enhanced specificity and reduced toxicity.

FUTURE RESEARCH DIRECTIONS

The epigenetics of brain disorders is a new, emerging topic with great potential for development of novel therapeutic strategies for intervention in the neurodegeneration and cognitive deficits associated with stroke and epilepsy. In the past decade, remarkable progress has been made in documenting a role for epigenetics in brain disorders such as seizures and ischemia. A case in point is the discovery that the gene-silencing transcription factor REST is activated in adult neurons in response to ischemia and seizures and orchestrates epigenetic remodeling and silen-cing of subsets of transcriptionally responsive target genes. Also of note, microRNAs are emerging as key modulators of post-transcriptional gene regulation critical to normal brain development. In the past several years, neural-specific microRNAs have been discovered; many of these act to

suppress networks of neuronal or non-neuronal genes. In addition, microRNAs can act in synaptodendritic compartments to regulate local translation of proteins important to synaptic plasticity and neuronal survival (Wang et al, 2010). Dysregulation of microRNAs in neurodegenerative diseases and in response to neuronal insults can contribute to neuronal death (Johnson et al, 2008; Hebert et al, 2009).

The past decade has witnessed an explosion of new information focused on site-specific epigenetic modifications of histone proteins, the concept that modifications at multiple sites can act synergistically to more dramatically promote transcriptional activation or repression ('multivalency'), the discovery of new transcription factors and other chromatin-modifying proteins and the mechanisms by which they activate or repress target genes. Moreover, extraordinary progress has been made in developing new technologies to examine epigenome-wide alterations in DNA methylation, histone modifications, and transcription factor occupancy in neurologic diseases and disorders.

Important commonalities between the mechanisms underlying ischemia and seizures have been discovered. In both disorders, the gene silencing transcription factor REST is activated in selectively vulnerable mature hippocampal neurons and orchestrates epigenetic remodeling and silencing of a subset of neuron-specific target genes. This observation raises the possibility that new therapeutic strategies used to treat one disorder such as epilepsy may also hold promise for treatment of other brain disorders such as stroke and possibly even neurodegenerative disorders such as Huntington's disease.

microRNAs represent an exciting alternative mode of post-transcriptional regulation in brain in response to neuronal insults such as ischemia or seizures. An important future direction would be to identify not only subsets of microRNAs that are up- or downregulated in these pathological conditions, but also the downstream targets of the microRNAs and their potential impact on neuronal survival or death. Armed with this knowledge, one could potentially develop novel therapeutic strategies to target/ modulate specific microRNAs. microRNAs are particularly interesting targets for pharmaceutical intervention in that a single microRNA can influence networks of neuronal and/ or nonneuronal genes (Fasanaro et al, 2010). One interesting application is the use of microRNAs as biomarkers for the diagnosis of neurological diseases. As an example, recent studies have identified specific microRNAs that are upregulated in the blood within hours after a heart attack and are currently investigating the use of these microRNAs as potential biomarkers for myocardial infarction (Salic and De Windt, 2012). microRNAs are widely used in several types of cancer, with one study identifying microRNAs that correlate with the clinical outcome of breast cancer patients (Wu et al, 2012). Currently, microRNAs are undergoing evaluation for possible clinical use as biomarkers for neurological diseases (de Planell-Saguer and Rodicio, 2011).

The brain expresses 70% of known microRNAs, each of which exhibits distinctive spatio-temporal expression (Cao et al, 2006; de Planell-Saguer and Rodicio, 2011). Dysregu-

lation of microRNA expression have been found in animal models of Alzheimer's disease (Hebert et al, 2009). If indeed altered levels of microRNAs are found to correlate with the severity of the disease, they could become valuable tools to diagnose this disease early and be able to administer treatment. There are several microRNAs that are altered in response to brain injury after stroke or seizures (Liu et al, 2010). In one study of young stroke patients, these profiles correlated with the type of stroke they suffered and the progress of recovery (Tan et al, 2009). These studies demonstrate that microRNAs, if specific and readily detectable at the appropriate times, could be powerful tools not only for the diagnosis, but also the treatment of neurological disorders.

The past decade has witnessed the application of drugs that target the epigenetic machinery in a variety of disorders. Although HDAC inhibitors and other drugs that target the epigenetic machinery are FDA-approved and in use clinically to treat patients with late-stage cancer and autoimmune disorders, use of these drugs to treat brain disorders such as ischemia and epilepsy is a new and burgeoning field. The finding that a single, acute injection of the HDAC inhibitor TSA administered to animals after an ischemic episode ameliorates neuronal injury has important clinical implications and suggests that HDAC inhibitors (Noh et al, 2012) may be a promising avenue for intervention in the neurodegeneration associated with ischemic stroke. Unsolved problems include the development of drugs that have the ability to penetrate the blood–brain barrier and ameliorate neuronal death and cognitive deficits, with minimal toxicity. This is important because although it might not be possible to prevent the initial stroke or seizure, if treated properly, drugs that target the epigenetic machinery may serve to limit or prevent further neuronal death, thereby limiting the severity of the insult.

Although compelling, these findings raise new questions. Does epigenetic remodeling promote susceptibility to neuronal insults such as seizures and ischemia, and/or do epigenetic modifications occur in response to neuronal insults? Findings from studies of neurological diseases with long-term, progressive neurodegeneration, such as Huntington's disease and Alzheimer's disease, indicate that epigenetic dysregulation may do both. Studies performed to date show that ischemic stroke promotes epigenetic dysregulation, which in turn promotes neuronal death. Recent findings add ischemic stroke and epilepsy to the growing list of disorders that involve dysregulation of chromatin-modifying proteins.

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DISCLOSURE

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