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Chromatin-Modifying Agents for Epigenetic Reprogramming and Endogenous Neural Stem Cell-Mediated Repair in Stroke

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Abstract

The recent explosion of interest in epigenetics and chromatin biology has made a significant impact on our understanding of the pathophysiology of cerebral ischemia and led to the identification of new treatment strategies for stroke, such as those that employ histone deacetylase inhibitors. These are key advances; however, the rapid pace of discovery in chromatin biology and innovation in the development of chromatin-modifying agents implies there are emerging classes of drugs that may also have potential benefits in stroke. Herein, we discuss how various chromatin regulatory factors and their recently identified inhibitors may serve as drug targets and therapeutic agents for stroke, respectively. These factors primarily include members of the repressor element-1 silencing transcription factor (REST)/neuron-restrictive silencer factor macromolecular complex, polycomb group (PcG) proteins, and associated chromatin remodeling factors, which have been linked to the pathophysiology of cerebral ischemia. Further, we suggest that, because of the key roles played by REST, PcG proteins and other chromatin remodeling factors in neural stem and progenitor cell (NSPC) biology, chromatin-modifying agents can be utilized not only to mitigate ischemic injury directly but also potentially to promote endogenous NSPC-mediated brain repair mechanisms.

Keywords

Cerebral ischemia; Chromatin-modifying agent; Epigenetic reprogramming; Histone modification; Polycomb group; Neural stem cell; REST/NRSF; Stroke

Rationale for Targeting Chromatin as a Strategy for Treating Stroke

After decades of relative obscurity, chromatin biology is now amongst the most exciting fields of basic science and translational research. Chromatin refers to the packaging of DNA within the cell nucleus. Its structural and functional dynamics serve as key “epigenetic” mechanisms that are responsible for modulating the expression of myriad protein-coding genes and emerging classes of non-coding RNAs (ncRNAs) encoded by the genome. Chromatin states also mediate the accessibility of DNA to diverse regulatory and functional proteins and ncRNAs. Thus, chromatin coordinates aspects of nearly every physiological and pathophysiological process. Targeting chromatin now represents one of the most promising avenues for the development of therapies for complex disorders and is actively being pursued in preclinical and clinical trials for the treatment of disease states such as cancer and neurodegeneration [1].

The pathobiology of cerebral ischemia is complex, and the molecular and cellular processes that are involved in the onset and progression of, and recovery from, stroke remain very active areas of investigation [2]. Nonetheless, preliminary studies have begun to highlight the roles played by specific chromatin regulatory factors and chromatin modifications both in promoting and preventing cerebral ischemia-induced neuronal death. For example, a recent landmark study showed that proteins involved in chromatin-related processes are selectively increased in the ischemia tolerant brain [3]. These factors were significantly enriched for the following gene ontology terms: nucleosome assembly, nucleosome organization, chromatin assembly, DNA packaging, chromatin assembly or disassembly, and protein–DNA complex assembly. These important observations suggest that an array of chromatin regulatory factors may be high value therapeutic targets in stroke. Histone deacetylase (HDAC) enzyme inhibitors are chromatin-modifying agents that have already shown great promise as treatments for stroke and for other neurological diseases [4, 5]. In fact, treatment with HDAC inhibitors decreases the extent of ischemic injury and improves functional outcomes in animal models of stroke by reprogramming neural cells and promoting the expression of genes and activation of pathways associated with survival rather than cell death [4]. Intriguingly, another mechanism of action for HDAC inhibitors in the ischemic brain may potentially be the stimulation of endogenous neural stem and progenitor cell (NSPC)-mediated regenerative responses. Indeed, a recent study showed that treatment with the HDAC inhibitor, sodium butyrate, after cerebral ischemia promoted NSPC proliferation, migration, and differentiation in the subventricular zone, hippocampal dentate gyrus, striatum, and frontal cortex [6].

In this article, we consider the roles of chromatin regulatory factors in the nervous system focusing on the potential for emerging classes of chromatin-modifying agents, beyond HDAC inhibitors, to serve as novel biological probes and treatments for stroke. Specifically, we discuss how chromatin-modifying factors are implicated in the pathophysiology of cerebral ischemia. These include members of the repressor element-1 silencing transcription factor (REST)/neuron-restrictive silencer factor macromolecular complex, such as the histone methyltransferases, G9a and SUV39H1, and the histone demethylase, LSD1, as well as polycomb group (PcG) proteins. We highlight the emergence of various small molecules and other agents that can modulate the activities of these factors (see Fig. 1 and Table 1). Further, we review the important roles played by chromatin regulatory factors in the

functions of NSPCs [7] and speculate that utilizing chromatin-modifying agents may be a viable strategy to stimulate endogenous NSPCs and enhance NSPC-mediated brain repair mechanisms. We believe that the need for new and improved research tools and therapies for stroke is clear and, despite the challenges, the promise of these observations and their implications is considerable.

Chromatin Structure and Function

Chromatin is comprised of DNA, histone proteins, and associated factors that together form the highly compacted superstructure of the genome present within the eukaryotic cell nucleus [8–10]. Chromatin exists along a continuum, from the level of a single gene to that of entire chromosomes. Dynamic changes in chromatin states can occur at multiple levels within this continuum, through local modifications or more global chromatin remodeling. Chromatin conformations may become more or less “open” or “closed” thereby modulating the accessibility of regulatory and functional sequences to each other and to a broad range of nuclear constituents, such as transcriptional coactivators and corepressors, transcription factors, and the transcriptional machinery itself. Euchromatin is loosely packaged and usually functionally active. In contrast, heterochromatin is more densely packaged and is inactive but nonetheless important for other purposes [9]. These chromatin states play important roles in mediating nuclear processes, such as gene activation and silencing as well as DNA replication and repair and the maintenance of genomic integrity.

The basic unit of chromatin is the nucleosome, which consists of DNA wrapped around an octamer of classical histone proteins (e.g., H2A, H2B, H3, H4). These histone proteins can be replaced with histone variants (e.g., H2A. Z), changing the biophysical and biochemical properties of the nucleosome and influencing diverse biological processes such as gene activation, chromosome segregation, and progression through the cell cycle [11]. DNA connecting nucleosomes is wrapped around linker histone proteins (e.g., H1). These histone proteins are subject to post-translational modifications including but not limited to acetylation, methylation, phosphorylation, ubiquitylation, SUMOylation, and ADP ribosylation that occur on residues of the N-terminal tails and at the core of these histone proteins. Each histone modification is catalyzed by a specific set of enzymes, for example, histone acetyltransferases and HDACs modulate histone acetylation and deacetylation, respectively [8]. Similarly, histone methylation and demethylation are catalyzed by histone methyl transferases, such as G9a and SUV39H1, and histone demethylases, such as LSD1. Intriguingly, these histone-modifying enzymes can also act on non-histone substrates [12]. For example, LSD1 can demethylate p53 at lysine (K) 370, thereby repressing its function [13].

Particular profiles of histone modifications indicate and promote specific biological functions. For example, trimethylated H3K27 (H3K27me3) and H3K9me3 are found in transcriptionally repressed chromatin. In contrast, H3K4me3 is found in transcriptionally active chromatin. Further, certain genes that are in a “poised” state for expression are associated with a “bivalent” histone signature consisting of both activating (i.e., H3K4me2/me3) and repressive (i.e., H3K27me3) histone modifications. All histone modifications are not, however, associated as clearly with particular biological functions. In fact, complex crosstalk between combinations of histone modifications is likely to form a hierarchical “code” that defines functional genomic regions, which promote or inhibit the binding of diverse factors containing certain interaction domains [8].

Effector molecules recognize, or “read,” specific histone modifications via particular protein domains (e.g., Tudor, PHD fingers, chromodomains, and bromodomains) [14]. These effectors bind to chromatin and subsequently promote structural and functional alterations.

For example, they can stabilize interactions between chromatin and large multimeric complexes, including those that promote nucleosome repositioning [e.g., SWItch/Sucrose NonFermentable (SWI/SNF)] or higher order chromatin remodeling (e.g., PcG) through further chemical modifications as well as those complexes encompassing RNA polymerase and related factors. These interactions can be heterogeneous and, further, single histone tails can engage in multiple interactions, thereby suggesting that histone and chromatin codes are multifaceted and complex. As such, reading and interpreting these codes seem to require the cooperative actions of many linked effector protein modules. In fact, many histone-, nucleosome-, and higher order chromatin-modifying enzymes and transcriptional modulators can act alone or as part of highly modular, macromolecular epigenetic regulatory complexes, such as the REST complex.

Chromatin-Modifying Enzymes as Potential Therapeutic Agents for Stroke

Members of the Repressor Element-1 Silencing Transcription Factor Macromolecular Complex

REST is a master transcriptional and epigenetic regulator that modulates the expression of a large repertoire of protein-coding [15–17] and non-coding [18, 19] genes including but not limited to those that are associated with repressor element-1/neuron-restrictive silencer element sequences. REST acts as a modular scaffold for the direct and indirect, context-specific recruitment of a broad array of transcriptional and epigenetic regulatory cofactors to these gene loci providing a high degree of flexibility in modulating chromatin structure and transcription at these sites.

REST binds to SIN3 proteins (i.e., SIN3A and SIN3B) at its N-terminus and to the corepressor for element-1 silencing transcription factor (CoREST) at its C-terminus [20]. SIN3 proteins are scaffolds and corepressors that, in turn, recruit HDAC1/2. Similarly, CoREST can recruit various factors, including HDAC1/2 and the histone H3K4 lysine demethylase, LSD1. REST also associates with the histone H3K9 methyltransferases, G9a and SUV39H1; the SWI/SNF chromatin remodeling complex components, BRG1, BAF170, and BAF57 and the high mobility group protein, BRAF35. In addition, REST can also interact with many other epigenetic and regulatory cofactors, including those involved in DNA methylation and ncRNA regulation.

By acting as a molecular platform to which these diverse factors can all be recruited, REST promotes dynamic histone modifications and higher order chromatin remodeling. These distinct REST complexes play key roles in modulating neural genes responsible for a broad range of developmental and homeostatic functions, including growth factors, axon guidance cues, ion channels, neurotransmitter receptors, synaptic vesicle proteins, components of the cytoskeleton, and elements of the extracellular matrix [21–23]. Further, REST is highly integrated with ncRNA networks through various complex feedback mechanisms [23]. REST has been implicated in the molecular pathophysiology of diverse nervous system disease states including ischemia [24, 25] as well as cancer [26–35], neurodegenerative and neurodevelopmental disorders [36–39], and epilepsy [40, 41].

A series of important studies have shown that members of the REST macromolecular complex are important in mediating neuronal responses to ischemia. For example, in an animal model of global cerebral ischemia, REST expression was upregulated in neurons destined to die, and knock down of REST rescued these post-ischemic neurons from cell death [25]. One effect of increased REST expression was to suppress the expression of the AMPA receptor subunit, GluR2 in post-ischemic neurons, through deacetylation of histone H3 and H4 within the GluR2 promoter region. This finding suggests that perturbations in AMPA receptor-mediated Ca^{2+} homeostasis are amongst the deleterious consequences of

REST upregulation in post-ischemic neurons. Another study showed that a functional outcome of increased REST availability was also to silence μ opioid receptor 1 (MOR-1) expression in post-ischemic neurons, through deacetylation of histone H3 and H4 and dimethylation of H3K9 in the MOR-1 promoter region [24]. REST-mediated HDAC1/2 activity is responsible for the deacetylation of these histones, and REST-mediated G9a activity is likely responsible for dimethylation of H3K9. In fact, it has previously been shown in other cell types that G9a promotes an increase in H3K9me2 under hypoxic conditions [42]. Moreover, the expression of LSD1, another member of the REST complex, is induced in the brain in response to transient global cerebral ischemia in a temporally and spatially selective manner that suggests it may play a role in delayed neuronal death [43].

In addition, SWI/SNF chromatin remodeling proteins associated with the REST complex have also been linked with cellular pathways that may be relevant in stroke pathophysiology. For example, BRG1 is implicated in mediating responses to ischemia in endothelial cells [44] and other cell types [45, 46]. BRG1, and to a lesser extent BAF170, are also involved in transforming growth factor β signaling pathways [47], which are associated with stroke [48, 49]. Further, BAF170 interacts with components of DNA damage response pathways [50] that are implicated in stroke [51]. Also, BRG1- and BAF170-containing SWI/SNF chromatin remodeling complexes include the activity-dependent neuroprotective protein, ADNP [52], which has neuroprotective effects in hypoxic-ischemic brain injury [53]. More generally, components of SWI/SNF chromatin remodeling complexes serve as regulators of hypoxia-inducible factor and are thus critical mediators of cellular responses to ischemia [54].

These observations strongly suggest that modulating the function of REST and members of the REST complex, either directly or indirectly, represent attractive approaches for mitigating neuronal death after ischemia.

Targeting Members of the Repressor Element-1 Silencing Transcription Factor Macromolecular Complex

Efforts aimed at discovering agents capable of inhibiting REST activity are currently underway [55–57]. These strategies have focused partly on screening chemical libraries to identify, or using structural and functional information to design, small molecules that can potentially interfere with the ability of REST to translocate into nucleus, bind to DNA, and interact with other members of the REST macromolecular complex. These approaches, coupled with REST reporter gene assays, have led to the discovery of 2-aminothiazole derivatives that have the potential to inhibit REST activity and to upregulate REST target gene expression. In addition, strategies employing RNA interference and decoy oligonucleotides or synthetic peptide nucleic acid oligomers have been explored, but these are currently more challenging to implement because of issues related to delivery and potential toxicities.

Compounds that can inhibit G9a and SUV39H1 histone methyl transferase activity have also been reported. The small molecule, BIX-01294, inhibits G9a and the fungal mycotoxin, chaetocin, inhibits SUV39H1 [58, 59]. BIX-01294 was identified in a chemical library screen for specific inhibitors of histone lysine methyltransferases using recombinant G9a as the target [59]. It is a diazepin–quinazolin–amine derivative that selectively and reversibly reduces G9a-mediated H3K9me2 in vitro. Recent studies of BIX-01294 structure activity relationships have led to the discovery of UNC0224, a more potent and selective G9a inhibitor [60]. High resolution X-ray crystallography was subsequently used for structure-based optimization of UNC0224 and resulted in the design of UNC0321, the most potent G9a inhibitor, which has a K_i of 63 pM [61].

Compounds that can inhibit LSD1 activity have also recently been identified. For example, because LSD1 belongs to the amine oxidase enzyme superfamily that includes monoamine oxidase (MAO), MAO inhibitors have been screened for the ability to inhibit LSD1 activity [62–64]. These studies revealed that MAO inhibitors, such as propargylamine and cyclopropylamines, are mechanism-based inactivators of LSD1. In particular, trans-2-phenylcyclopropylamine (2-PCPA, tranlycypromine) is a time-dependent, irreversible inhibitor of LSD1 with a K_i of 242 mM that is commercially available under the brand name Parnate® [63]. Further, two 2-PCPA derivatives, trans-2-pentafluorophenylcyclopropylamine and S1201, have successfully been designed to more specifically inhibit LSD1 [65]. Indeed, S1201 exhibits stronger LSD1 inhibition than 2-PCPA in demethylation assays, with a K_i value less than 1 μ M. Phenethylhydrazine is another MAO inhibitor, available under the brand name Nardil®, that is a potent LSD1 inhibitor. In addition to these small molecules, specific polyamine analogs can also inhibit LSD1 activity. In fact, polyamines play key roles in DNA stabilization, chromatin regulation, and RNA processing, and specific PAHAs (polyaminohydroxamic acids) and PABAs (polyaminobenzamides) polyamine analogs are known to inhibit HDACs. A recent study also demonstrated that biguanide, bisguanidine, and oligoamine classes of polyamine analogs are potent inhibitors of LSD1 [66, 67].

Polycomb and Trithorax Group Proteins

PcG and trithorax (TrxG) group proteins form combinatorially assembled, multi-subunit complexes with respective inhibitory and activating histone modification and associated higher order chromatin remodeling activities. PcG proteins form polycomb repressor complexes (PRC) associated with gene silencing. At least three different PRCs are found in mammals—PRC1, PRC2, and PhoRC. PRC1 has four core subunits, including one from each of the following families: BMI1, PHC, CBX, and RING1. More than 60 possible combinations exist. In contrast, PRC2 is comprised of EED, SUZ12, and either the EZH1 or EZH2 histone methyl transferase, the catalytic subunits of the complex. These histone methyl transferases are required for the initiation of gene silencing through the di- and tri-methylation of H3K27. More recently, it has been shown that JARID2 is a non-canonical subunit of PRC2 [68]. TrxG proteins assemble into complexes consisting of MLL1, WDR5, RBBP5, and ASH2L that promote gene activation. MLL1, the catalytic subunit, is an H3K4 methyltransferase. These PRC and TrxG complexes are also functionally interrelated with various interactors, including those involved in DNA methylation and ncRNA regulation, in a cell type- and gene-specific manner. Each complex is responsible for regulating hundreds of genes and controls a range of cellular physiological and pathophysiological processes including but not limited to differentiation and oncogenesis; however, the particular biological functions associated with distinct complex assemblies have yet to be elucidated.

Intriguingly, one recent study has significantly advanced our understanding of the roles played by PcG proteins in stroke including, specifically, their potential to promote ischemic tolerance in the setting of ischemic preconditioning [3]. This proteomic analysis of brain tissues found that the hallmark of ischemic tolerance is an increase in the levels of PcG proteins, particularly, BMI1 and SCMH1 as well as histone H2A and H2B variants. Remarkably, this study demonstrated that direct knock down of these PcG proteins abrogates ischemic tolerance, whereas over expression of BMI1 and SCMH1 induces ischemic tolerance without ischemic preconditioning. PRC2 and its components may play similar roles [3]. How might these factors be operating? Potential mechanisms include the modulation of mitochondrial function and antioxidant defenses and cell cycle machinery. In fact, BMI1 is thought to regulate genes involved in these pathways in various cell types including neurons, by repressing p53 levels and associated pro-oxidant activity and suppressing apoptosis [69]. Further, PcG proteins modulate gene loci linked to stroke risk,

such as the *INK4b-ARF-INK4a* locus, which encodes the INK4 cyclin-dependent kinase inhibitors, p15(INK4b) and p16(INK4a), and the tumor suppressor, ARF [70].

Notably, this gene locus is also regulated by other epigenetic regulators including SWI/SNF chromatin remodeling complexes [71] and CoREST [72], highlighting the functional interrelationships between these different factors. Indeed, it has been suggested that PRC1 and PRC2 are even recruited to their genomic sites of action by REST [38, 73, 74]. PcG proteins and the REST complex can also be corecruited to genomic sites by particular ncRNAs [75]. These thought-provoking observations underscore the mechanistic links that may exist between PcG proteins and the REST complex and draw attention to functional relationships that may play a role in the pathophysiology of cerebral ischemia.

Targeting PcG and TrxG Proteins

Agents that affect PcG and TrxG proteins have been characterized. The *S*-adenosylhomocysteine hydrolase inhibitor 3-deazaneplanocin (also known as DZNep) effectively reduces cellular levels of the PRC2 components, EZH2, SUZ12, and EED; inhibits H3K27 methylation; and upregulates PRC2 target genes [76]. Similarly, dietary omega-3 polyunsaturated fatty acids (PUFAs) may influence the expression and activity of EZH2 [77]. A recent study showed that treatment with omega-3 PUFAs downregulated EZH2, probably through post-translational mechanisms decreased H3K27me3 and upregulated EZH2 target genes. This study implies that the downregulation of EZH2 may be one mechanisms of action explaining the multiple beneficial effects of omega-3 PUFAs in stroke [78]. In addition, compounds that can affect MLL1 expression and function have been reported. For example, exposure to the mycotoxin, deoxynivalenol, promotes overexpression of MLL1 [79]. By contrast, radicicol reduces levels of MLL1 and binding of TrxG complexes to DNA resulting in downregulation of TrxG target genes [80]. Interestingly, these effects of radicicol are mediated by its ability to inhibit HSP90 and, in fact, a functional interaction between HSP90 and TrxG proteins is required for activating TrxG target gene expression [80]. These observations suggest that other HSP90 inhibitors, such as geldanamycin, may also have the potential to inhibit TrxG activity. Also, the interaction with WDR5 is important for the H3K4 methyltransferase activity of MLL1. A recent study delineated the essential elements in MLL1 required for the binding of WDR5 [81]. It thereby provided a strategy for designing compounds to inhibit MLL1 activity by targeting and disrupting the MLL1 and WDR5 interaction. Further, it reported that two 3-mer peptides, Ac-ARA-NH₂ and Ac-ART-NH₂, designed to inhibit the interaction between MLL1 and WDR5, did so with *K_i* values of 120 and 20 nM, respectively.

Chromatin-Modifying Agents for Endogenous Neural Stem and Progenitor Cell-Mediated Brain Repair

Neural Stem and Progenitor Cell (NSPCs) can give rise to neurons, oligodendrocytes, and astrocytes—the three primary cell types of the mature brain—and are distributed throughout specialized niches of the adult brain [82]. After cerebral ischemia, pools of these endogenous NSPCs have the potential to expand, migrate to injured regions, differentiate into mature cell types including neurons and promote neural repair mechanisms [83–85]; however, the scope of endogenous NSPC activation after injury is very limited. One of the key challenges in regenerative medicine is, therefore, to identify strategies for stimulating these endogenous NSPCs to engage more robustly in tissue remodeling and repair after injury. Many studies have focused on promoting NSPC self-renewal, proliferation, migration, survival, lineage commitment, terminal differentiation, and neural network integration by utilizing growth factors and cytokines [83, 84]. These approaches have achieved varying degrees of success.

Intriguingly, detailed in vitro and in vivo experiments have established the importance of histone acetylation for neural lineage specification and maturation and also shown that administration of HDAC inhibitors can modulate these processes [7]. For example, the HDAC inhibitor, valproic acid, promotes neuronal differentiation of multipotent adult hippocampal neural progenitor cells by inducing pro-neuronal genes (e.g., *NeuroD*, *Ngn1*, *Math1*, and *p15*) [86, 87]. Similarly, the HDAC inhibitor, trichostatin A, prevents the progression of oligodendrocyte (OL) progenitors into post-mitotic OLs by suppressing OL-specific gene expression programs. Further, it can reprogram specified OL developmental species into a multipotent state that is receptive to both neurogenic and astrogliogenic differentiation signals by preventing the downregulation of NSPC genes including the critical transcription factor, *Sox2*. These and related observations have led to speculation and, subsequently, to verification that HDAC inhibitors can enhance NSPC proliferation, migration, and differentiation in the brain after ischemic injury [6].

Additional studies have begun to characterize how chromatin regulatory factors beyond HDACs, such as other members of the REST, PcG, and TrxG complexes, are similarly responsible for orchestrating developmental gene expression and modulating NSPC functions [7]. For example, we recently demonstrated that the REST and CoREST regulatory networks encompass neural genes, which mediate NSPC maintenance, lineage restriction, neuronal and glial fate specification, and terminal differentiation and are highly integrated with ncRNA networks [21–23]. Moreover, LSD1 has been shown to regulate NSPC proliferation [88]. The PcG protein, BMI1, mediates NSPC self-renewal and proliferation [89]. Correspondingly, the TrxG protein, MLL1, is essential for neurogenesis but not gliogenesis [90]. Further, the PcG protein EZH2 is involved in the differentiation of NSPCs into OLs [91]. Because of these provocative observations, we suggest, herein, that targeting these factors and utilizing the emerging classes of chromatin-modifying agents discussed above represent innovative strategies for stimulating endogenous NSPCs to recapitulate neural developmental processes, thereby providing more effective treatment modalities for stroke as well as other nervous system disorders.

These approaches are consistent with recent reports describing compounds that have the ability to regulate embryonic stem cell fate and to reprogram somatic cells, including NSPCs [92, 93], and with more general conjectures about the potential applications for chromatin-modifying agents in regenerative medicine. Indeed, preliminary studies have shown that BIX-01294, a small molecule inhibitor of G9a, can be used in certain contexts to promote reprogramming of somatic cells into induced pluripotent stem cells [94].

Perspectives

Cerebral ischemia leads to widespread alterations in gene expression that ultimately result in inflammation and neural cell death. Attaining a better understanding of the molecular mechanisms involved in mediating these gene expression changes is an important, and perhaps even a necessary, step for the development of novel therapies for acute stroke. In the post-genomic era, increasingly sophisticated studies such as those employing ultra high throughput DNA and RNA sequencing have revealed that epigenetic mechanisms play critical roles in gene regulation. These highly interconnected, cell type-specific, and environmentally responsive processes include DNA methylation, chromatin regulation, ncRNA dynamics, and nuclear reorganization [1]. Major efforts such as the Human Epigenome Project have recently been launched in order to catalog and interpret the complex and evolving profiles of merely a subset of these epigenetic factors and their functions in health and disease [95].

Epigenetic mechanisms have emerged as principal mediators of nervous system disorders, from neurodevelopmental and neurodegenerative diseases to cancer [1]. Stroke is no exception. The roles played by chromatin regulation in the pathophysiology of cerebral ischemia are increasingly being characterized (see above). In fact, stress pathways modulating neural cell viability are clearly modulated by chromatin structure, as evidenced by the “protective” generalized transcriptional repression that is associated with ischemic preconditioning and mediated by repressive PcG proteins [3]. Chromatin regulation is also a crucial mechanism responsible for the execution of neural developmental processes, including NSPC-mediated neuronal and glial lineage elaboration. The range of observations we have highlighted provides strong evidence for supporting efforts aimed at the further development of existing and novel compounds targeting chromatin-modifying factors in order to treat stroke; however, many biological questions and technical barriers lie ahead in order to carry out proof-of-concept studies and to translate these ideas into real-world therapeutics.

Several fundamental biological questions have yet to be answered regarding chromatin biology, in general, and its function (or dysfunction) in stroke. For example, if chromatin remodeling complexes can potentially target hundreds, if not thousands, of genes then what are the mechanisms responsible for their genomic site-selective recruitment and context-specific effects? What specific interactions exist between chromatin and diverse classes of novel and emerging short [e.g., microRNAs, PIWI-interacting RNAs, small nucleolar RNAs (snoRNAs), small RNAs derived from snoRNAs, transcription initiation RNAs, promoter-associated small RNAs, termini-associated small RNAs, and enhancer RNAs] and long ncRNAs? How do these functional relationships influence changes in gene expression that occur in response to cerebral ischemia? Do “epigenetic memory” states that are mediated by complex chromatin dynamics play a role in the pathophysiology of cerebral ischemia? What are the effects of chromatin regulatory factors on the multiplicity of stroke comorbidities and risk factors, such as hypertension, diabetes, and atherosclerosis? Can biomarkers for stroke risk, onset, and progression be developed that are based on chromatin structure and dynamics?

Significant technical barriers also exist for designing and delivering chromatin-modifying agents to the brain. Can these agents be engineered to selectively modulate a particular chromatin regulatory factor and be optimized to promote particular gene expression programs (i.e., those associated with survival in cells at risk and/or with neural repair in NSPCs)? Can these agents be delivered successfully to the target neural cells (i.e., cells at risk and/or NSPCs) without off-target effects in the brain, vascular, immune, and other systems to minimize toxicities, perhaps through emerging neurointerventional approaches?

Despite these myriad challenges, however, progress in the development of new tools to modulate chromatin regulatory factors will, no doubt, help to elucidate epigenetic mechanisms that may play a role in stroke, to provide a basis for novel therapeutic approaches to correct gene expression in ischemic neural cells, and possibly to augment endogenous NSPC-mediated repair mechanisms.

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A. Modulate expression levels of complex components **B. Disrupt interactions between complex components** **C. Disrupt catalytic activity of complex components** **D. Inhibit binding of complex to DNA**

Fig. 1.
Illustration depicting the potential mechanisms of action for different chromatin-modifying agents

Table 1

Examples of small molecules and other agents for modulating the expression and function of chromatin regulatory factors with roles in cerebral ischemia

Chromatin regulatory factor	Chromatin-modifying agents
REST	• 2-Aminothiazole derivatives
G9a	• BIX-01294 • UNC0224 • UNC0321
SUV39H1	• Chaetocin
LSD1	• Propargylamine • Cyclopropylamine derivatives (trans-2-phenylcyclopropylamine, trans-2-pentafluorophenylcyclopropylamine, and S1201) • Phenethylhydrazine • Biguanide, bisguanidine, and oligoamine analogs
PcG proteins (EZH2, SUZ12, and EED)	• 3-Deazaneplanocin • Omega-3 polyunsaturated fatty acids
TrxG proteins (MLL1 and WDR5)	• Deoxynivalenol • Radicol • 3-Mer peptides (Ac-ARA-NH ₂ and Ac-ART-NH ₂)