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Balancing Histone Methylation Activities in Psychiatric Disorders

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Abstract

Alterations in histone lysine methylation and other epigenetic regulators of gene expression contribute to changes in brain transcriptomes in mood and psychosis spectrum disorders, including depression and schizophrenia. Genetic association studies and animal models implicate multiple lysine methyltransferases (KMTs) and demethylases (KDMs) in the neurobiology of emotion and cognition. Here, we review the role of histone lysine methylation and transcriptional regulation in normal and diseased neurodevelopment and discuss various KMTs and KDMs as potential therapeutic targets in the treatment of neuropsychiatric disease.

Epigenetics and Mental Illness

Schizophrenia and depression are major psychiatric disorders that lack consensus neuropathology and, in a large majority of cases, a straightforward genetic risk architecture. Furthermore, many patients on the mood and psychosis spectrum show an incomplete response to conventional pharmacological treatments which are mainly aimed at monoamine signaling pathways in the brain (Box 1).

Box 1

Schizophrenia and Depression

Schizophrenia affects 1% of the general population and typically begins during young-adult years, although cognitive disturbances could be evident much earlier. The disease is, in terms of genetics and etiology, highly heterogeneous, and increasingly defined as different and partially independent symptom complexes: (i) psychosis with delusions, hallucinations and disorganized thought; (ii) cognitive dysfunction including deficits in attention, memory and executive function; and (iii) depressed mood and negative symptoms including inability to experience pleasure (anhedonia), social withdrawal and poor thought and speech output [42]. Currently prescribed antipsychotics, which are mainly aimed at dopaminergic and/or serotonergic receptor systems, exert therapeutic effects on psychosis in approximately 75% of patients. However, it is the cognitive impairment which is often the more disabling and persistent feature of schizophrenia [42]. Currently there are no established pharmacological treatments for this symptom complex. However, given that cognitive dysfunction is an important predictor for long-

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term outcome, this area is considered a high priority in schizophrenia research, as reflected by initiatives combining efforts from government agencies, academia and industry, including MATRICS (the Measurement and Treatment Research to Improve Cognition in Schizophrenia) [42].

Affective disorders as a group show, in terms of genetic risk architecture, some overlap with schizophrenia. For example, rare structural variants, including the balanced translocation at the Disrupted-in-Schizophrenia 1 (DISC-1) locus (1q42) or the 22q11 deletion are, in different individuals, associated with either mood disorder or schizophrenia [81,82].

Depression, including its more severe manifestation, major depressive disorder which has a lifetime risk of 10–15% for the U.S. general population, is associated with excessive sadness, anhedonia, negative thoughts, and neurovegetative symptoms including changes in sleep pattern and appetite [1]. The disorder, which in more severe cases is accompanied by delusions, hallucinations and other symptoms of psychosis, often takes a chronic and recurrent course. Conventional antidepressant therapies primarily target monoamine metabolism and reuptake mechanisms at the terminals of serotonergic, noradrenergic and dopaminergic neurons. Unfortunately, up to 40% of cases show an insufficient response to these pharmacological treatments [1]. In addition, many antipsychotic and antidepressant drugs have significant side effect burden, including weight gain, diabetes and metabolic defects, extrapyramidal symptoms and sexual dysfunction [83,84].

However, there is evidence that dysregulated gene transcription, indicative of compromised neural circuitry, contributes to disordered brain function in psychosis and mood spectrum disorder [1,2]. While no single gene transcript is consistently affected, alterations in RNA levels contribute to defects in GABAergic inhibitory neurotransmission and more generally, synapse organization and function, metabolism and mitochondrial functions, and oligodendrocyte pathology [3–5]. While a number of transcriptional and post-transcriptional mechanisms may contribute to these changes, chromatin-associated proteins and epigenetic regulators invoked in sustained alterations of gene expression and function (Box 2) could play a critical role in the pathophysiology, or treatment of mental illness [6,7]. Indeed, there is evidence that changes in acetylation of histone lysine residues, which are broadly associated with active gene expression [8] and considered a potential therapeutic target for cancer and other medical conditions [9], also impact gene expression patterns in the brain and thereby influence emotional and cognitive functions. For example, mice or rats exposed to systemic treatment, or localized intracranial injections of class I/II histone deacetylase inhibitors (HDACi) exhibit behavioral changes reminiscent of those elicited by conventional antidepressant drugs [10–13]. The short chain fatty acid derivative valproic acid, widely prescribed for its mood-stabilizing and anticonvulsant effects, induces brain histone hyperacetylation at a select set of gene promoters when administered to animals at comparatively high doses [14]. Conversely, overexpression of selected HDACs in neuronal structures implicated in the neurobiology of depression, including the hippocampus, elicit a pro-depressant behavioral phenotype [12]. Similarly, animals treated with class I/II HDACi often show improved performance in learning and memory paradigms and furthermore, drug-induced inhibition or activation of class III HDAC (also known as sirtuins) elicits changes in motivational and reward-related behaviors [15]. Therefore, the orderly balance between histone acetyl-transferase and deacetylase activities is critical for cognitive performance and synaptic and behavioral plasticity [16]. Likewise, However, HDACs interfere with acetylation of many non-histone proteins in the nucleus and cytoplasm [16], and moreover, some of these drugs carry a significant side effect burden [9]. Therefore, in light of the emerging role of epigenetic mechanisms in the neurobiology of these and other

psychiatric conditions [6], the therapeutic potential of chromatin modifying drugs, other than the HDACi, warrants further investigations. This review will focus on histone lysine methylation, one of the most highly regulated chromatin markings in brain and other tissues. Multiple methyltransferases (KMTs) and demethylases (KDMs) were recently implicated in emotional and cognitive disorders (Fig. 1), and these types of chromatin modifying enzymes could emerge as novel targets in the treatment of mood and psychosis spectrum disorders.

Box 2

Epigenetic regulators and chromatin structure and function

Epigenetics, in the broader sense, applies both to dividing and postmitotic cells, and refers to a type of cellular memory that involves sustained changes in chromatin structure and function, including gene expression, in the absence of DNA sequence alterations (For in depth discussion, see [85]). Chromatin is essentially a repeating chain of nucleosomes comprised of genomic DNA wrapped around an octamer of core histones H2A/H2B/H3/H4. The histone proteins are intensely decorated with epigenetic information, with more than 70 (amino acid) residue-specific sites subject to various types of post-translational modifications (PTM). These include lysine (K) acetylation, methylation and poly ADP-ribosylation, arginine (R) methylation, and serine (S), threonine (T), tyrosine (Y) and histidine (H) phosphorylation [86]. In addition, a subset of the histone H2A, H2B and H4 lysines are covalently linked to the small protein modifiers ubiquitin and SUMO [87,88]. Finally, epigenetic markings in genomic DNA include 5-methyl-cytosine and the related form, 5-hydroxy-methyl-cytosine [85]. These DNA and nucleosomal histone markings define the functional architecture of chromatin (see main text).

Proteins associated with methylation and other histone PTM are typically defined either as ‘writers’, ‘erasers’ or ‘readers’, essentially differentiating between the process of establishing or removing a mark as opposed to providing a docking site for chromatin remodeling complexes that regulate transcription, or induce and maintain chromatin condensation [18,86,89]. As it pertains to the brain, especially in the context of neuropsychiatric disease, a substantial body of knowledge has been generated for a select set of site-specific (K) methyltransferases and demethylases (Fig. 1A). In contrast, many PTMs are recognized by large numbers of reader proteins [90], but to date only very few of these readers have been explored in the brain. To mention just two examples, there are approximately 75 reader proteins specifically associated with histone H3-trimethyl-lysine 4 (H3K4me3), including several components of the SAGA complex ascribed with a key role for transcriptional initiation at RNA polymerase II target genes [90]. In contrast, H3K9me3, generally considered a repressive mark, provides a central hub for heterochromatin (associated) proteins including several members of the HP1 family and zinc finger domain containing molecules [90]. There is additional complexity because pluripotent stem cells and additional cell types decorate many of their promoters with ‘bivalent domains’ which include both open chromatin-associated (methylated H3K4 and H3/H4 acetylation) and repressive (methylated H3K27) marks [91,92].

Histone methylation – an overview

The methylation of lysine and arginine residues, like other histone PTM, define chromatin states and function [8,17]. To date, more than 20 methyl-marks on K and R residues have been described [18,19]. As it pertains to the lysines, the majority of studies focused on the regulation and methylation-related functions of six specific sites: H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20 [18]. For H3K4 and H3K9/K27, there is additional complexity because specific information is also conveyed (i) for H3K4, the unmethylated lysine effectively serving as a DNA methylation signal [20,21], and (ii) for H3K9/K27 acetylation

as an alternative PTM [22,23] (Fig. 1A). For the aforementioned H3/H4 residues, specific biological functions and their interrelations with functional chromatin states, including transcriptional initiation and elongation, heterochromatic silencing and other mechanisms, have been described for the trimethyl-, and for some of the mono- and dimethyl-modifications (Representative examples are provided in Fig. 1B. See ref [17] for a detailed description of the histone methylation code and its relation to other types of histone PTM).

The following examples further illustrate the complex regulation of histone lysine methylation. Monomethylation of histone H3-lysine 4 (H3K4me1) plays an important role in neuronal activity-induced transcription at enhancer sequences [24], but the related forms, H3K4me3/2 are primarily found at the 5′ end of genes, with H3K4me3 mostly arranged as distinct and sharp peaks within 1–2Kb of transcription start sites. The H3K4me3 mark provides a docking site at the 5′ end of genes for chromatin remodeling complexes that either facilitate or repress transcription [25]. Furthermore, mono-methyl-H4-K20 shows strong positive correlation with gene expression at promoters enriched with CpGs, which contrasts to the trimethylated form of the same residue which generally is associated with repressed chromatin [23]. Taken together, these examples illustrate that even closely related histone lysine methylation markings are potentially associated with very different chromatin states.

To date, H3K4, H4K9, H3K27 and H4K20 methylation signals were measured at specific loci and genome-wide in human brain, essentially confirming that each of these epigenetic markings defines the same type of chromatin as in the peripheral tissues or animal brain [26–30]. Interestingly, a subset of psychotherapeutic drugs including the mood-stabilizer valproate, the atypical antipsychotic clozapine and some monoamine oxidase inhibitors and stimulant drugs interfere with brain histone methylation (Table 1).

Molecular mechanisms of histone (lysine) methylation

A complex system of site-specific methyltransferases, which transfer the methyl-group of S-Adenosyl-Methionine (SAM) to lysine residues, has evolved in the vertebrate cell. There are an estimated 70 human genes harboring the ***S****u(var)3–9*, ***E****nhancer of Zeste*, ***T****rithorax* (SET) domain, which spans approximately 130 amino acids essential for KMT enzymatic activity [31]. The only known exception is the H3K79-specific methyltransferase, KMT4/DOT1L [31,32], which lacks a SET. Each of the histone K residues discussed above is the preferential target of a distinct set of methyltransferase proteins (Fig. 1A)[19]. Of note, these histone-modifying enzymes are thought not to access histone substrates directly unless recruited by DNA-bound activators and repressors, a mechanism which could target each methyltransferase to a highly specific set of genomic loci [19].

An equally complex system exists for the site-specific lysine demethylases (Fig. 1A). There are at least two different mechanisms for active histone demethylation. The first enzyme type, represented by lysine-specific demethylase 1 (LSD1/KDM1A), contains an amine oxidase domain and requires flavin adenine dinucleotide (FAD) as a cofactor to demethylate di- and mono-methylated lysines. LSD1 and its homologue, LSD2, are primarily H3K4 demethylases, albeit depending on species and context, and activity against H3K9 also has been described [18]. Interestingly, monoamine oxidase inhibitors (MAOi) such as tranylcypromine or phenelzine — powerful antidepressants that exert their therapeutic effects mainly by elevating brain monoamine levels through inhibition of MAO-A/B — also block LSD1 type histone demethylases [18]. While LSD1 is thought to regulate histone methylation at promoters, LSD2 is bound to transcriptional elongation complexes and removes H3K4 methyl markings in gene bodies, thereby facilitating gene expression by reducing spurious transcriptional initiation outside of promoters [33]. The second type of

demethylase, which in contrast to LSD1/LSD2 is capable of demethylating trimethyl markings, involves Fe^{2+}-dependent dioxygenation by Jumonji-C (JmJC) domain-mediated catalysis [18]. Given that each of the KMTs and KDMs described has a different combinatorial set of functional domains and (protein) binding partners [18,34], it is likely that the various site-specific methyltransferases and demethylases are largely non-redundant in function.

KMTs and KDMs with a role in cognition and neuropsychiatric disease

An increasing number of KMTs and KDMs are implicated in neurodevelopment and major psychiatric diseases (marked in red in Fig. 1A).

H3K4

The first histone lysine methyltransferase explored in the nervous system was KMT2A/MLL1, a member of the mixed-lineage leukemia (MLL) family of molecules. Mice heterozygous for an insertional (lacZ) loss-of-function *Mll1* mutation show distinct abnormalities in hippocampal plasticity and signaling [35], in conjunction with defects of learning and memory [36]. Of note, the hippocampus, and other portions of the forebrain including prefrontal cortex and ventral striatum, are frequently implicated in the neural circuitry of mood and psychosis spectrum disorders [1]. Furthermore, conditional deletion of *Mll1* resulted in defective neurogenesis during the early postnatal period [37]. While the full spectrum of MLL1 target genes in neurons and glia awaits further investigation, dysregulated expression of certain transcription factors such as DLX2, a key regulator for the differentiation of forebrain GABAergic neurons (which are essential for inhibitory neurotransmission and orderly synchronization of neural networks) [38], may contribute to the cognitive phenotype of the *Mll1* mutant mice. These observations may be relevant for the pathophysiology of schizophrenia, because some patients show in the prefrontal cortex a deficit in H3K4-trimethylation and gene expression at a subset of GABAergic promoters, including *GAD1* encoding a GABA synthesis enzyme [28]. While the timing and age-of-onset for this ‘molecular lesion’ remains unknown, it is of interest that in the normal PFC, H3K4 methylation at the site of GABAergic genes progressively increases during the transition from fetal period to childhood to adulthood [28]. The epigenetic vulnerability of the *Gad1* promoter during such prolonged developmental periods is further emphasized by recent animal studies demonstrating that *Gad1*-DNA methylation and histone acetylation are heavily influenced by the level of maternal care in the neonatal period/pre-weanling period [39].

There is additional evidence that epigenetic fine-tuning of the brain’s H3K4 methyl-markings is critical for orderly neurodevelopment. Of note, loss-of-function mutations in *KDM5C/JARID1C/SMCX*, an X-linked gene encoding a H3K4 demethylase, have been linked to mental retardation [40] and autism spectrum disorders [41]. The *KDM5C* gene product operates in a chromatin remodeling complex together with HDAC1/2 histone deacetylases and the transcriptional repressor REST, thereby poising neuron-restrictive silencer elements for H3K4 demethylation and decreased expression of target genes including synaptic proteins and sodium channels [40]. However, because this study was conducted with the HeLa cell line, it remains to be determined whether similar mechanisms operate in the nervous system.

In addition to its role in neurodevelopment, MLL-mediated H3K4 methylation could play a potential role for the treatment of psychosis. The atypical antipsychotic clozapine, which has a somewhat higher therapeutic efficacy when compared to conventional antipsychotics that function primarily as dopamine D2 receptor antagonists [42], upregulates H3K4 tri-methylation at the *Gad1/GAD1* GABA synthesis enzyme gene promoter. These effects were

not mimicked in dopamine receptors D2/D3 (*Drd2/3*) compound null mutant mice, suggesting that blockade of dopamine D2-like receptors is not sufficient for clozapine-induced H3K4 methylation [28]. In the human PFC, *GAD1*-associated H3K4 methylation was increased in subjects exposed to clozapine, as compared to subjects treated with conventional antipsychotics. Conversely, mice heterozygous for the H3K4-specific KMT, mixed-lineage leukemia 1 (MLL1), exhibited decreased H3K4 methylation at brain *Gad1* [28]. Therefore, it is possible that MLL1, which is highly expressed in GABAergic and other neurons of the adult cerebral cortex [28], will in the future emerge as a novel target for the treatment of psychosis. Questions that remain to be resolved include (i) the molecular pathways linking clozapine — a drug that impacts dopaminergic, serotonergic, muscarinic and other signaling pathways — to MLL1-mediated histone methylation, and (ii) whether or not the clozapine-induced changes in H3K4 methylation are restricted to GABAergic gene promoters or, alternatively, the reflection of more widespread epigenetic changes throughout the genome. Of note, clozapine's effects on H3K4 methylation require intact brain circuitry and cannot be mimicked in cultured neurons differentiated from forebrain progenitor cells [43]. This finding is in good agreement with the recent observation that some of clozapine's therapeutic effects require an intact serotonergic system, particularly its presynaptic components [44].

H3K9

The 9q34 subtelomeric deletion syndrome, which includes mental retardation and other developmental defects, is caused by deleterious mutations and haploinsufficiency of euchromatin histone methyltransferase 1 (EHMT1, also known as GLP and KMT1D) [45]. This gene encodes a H3K9-specific methyltransferase that operates in a multimeric complex that includes its closest homologue, G9a/KMT1C, and additional H3K9-specific HMTs [46]. Studies in mutant mice suggest that the GLP/G9a complex is important for suppression of non-neuronal and progenitor genes in mature neurons, and loss of this complex has deleterious effects on cognition and other higher brain functions [47]. Furthermore, G9a-mediated H3K9 methylation events within the reward circuitry, including the ventral striatum, are critical intermediates for the long-term effects of cocaine on reward behavior and neuronal morphology [48]. This would suggest that GLP/G9a, and proper regulation of H3K9 levels, is important for orderly brain function both in developing and mature brain.

Furthermore, changes in motivational and affective behaviors could be elicited by overexpression of the H3K9-HMT, SET domain bifurcated 1 (KMT1C/SETDB1/ESET), in adult forebrain neurons [49]. Interestingly, SETDB1 occupancy in neuronal chromatin is highly restricted, and may be confined to less than 0.75% of annotated genes [49]. However, among these are several NMDA and other ionotropic glutamate receptor subunit genes, including *Grin2a/b (Nr2a/b)*[49]. Mild to moderate inhibition of NMDA receptor-mediated (including Grin2b) neurotransmission elicits a robust improvement of depressive symptoms in some mood disorder patients [50], and, indeed, SETDB1-mediated H3K9 methylation and repressive chromatin remodeling at the *Grin2b* locus was associated with antidepressant-like behavioral phenotypes in the *Setdb1* transgenic mice [49]. Of note, NMDA receptor antagonists, including GRIN2B-specific drugs, elicit significant therapeutic benefits even in subjects who failed multiple trials of selective serotonin reuptake inhibitors (SSRI) and other conventional antidepressants [50]. However, drugs directly acting at the NMDA receptor site have an unfavorable side effect profile, and therapeutic strategies aimed at SETDB1 expression and activity may therefore provide an alternative strategy.

Interestingly, mice with a genetic ablation of *Kap1*, encoding the SETDB1 binding partner KRAB-associated protein 1, also known as TRIM28/TIF1b/KRIP1)[51], show increased anxiety and deficits in cognition and memory [52], which are phenotypes that are broadly opposite from those observed in mice with increased *Setdb1* expression in brain [49]. These

findings further speak to the therapeutic potential of the Kap1-Setdb1 repressor complex in the context of neuropsychiatric disease.

Finally, the H3K9-specific demethylase, KDM3A/Jmjd1A, showed increased H3K9-methylation at its own promoter in the ventral striatum of mice exposed to social defeat (a type of stressor associated with a depression-like syndrome in these animals), while mice that were treated with a conventional antidepressant or that were resilient to this type of stress did not show changes in KDM3A promoter methylation [53]. While it is unclear whether KDM3A or some other demethylase acitivity is altered in the depressed animals, the same study [53] reported widespread repressive histone methylation changes, including increased dimethyl-H3K9 and methylated H3K27 at hundreds of gene promoters in stress susceptible animals, which further emphasizes the importance of these PTMs for the epigenetics of mood disorder.

H3K27

The H3K27-selective methyltransferase, KMT6A, also known as Enhancer of zeste homolog2 (EZH2), is associated with the polycomb repressive chromatin remodeling complex 2 (PRC2) [54], and essential for cortical progenitor cell and neuron production. Consequently, loss of EZH2 function is associated with severe thinning of the cerebral cortex and a disproportionate loss of neurons residing in upper cortical layers I–IV [55]. Likewise, the H3K27-specific demethylase, JMJD3, is important for neurogenesis and neuronal lineage commitment [56]. Furthermore, H3K27 methylation is dynamically regulated in mature brain and involved in the neurobiology of major psychiatric disease. For example, changes in expression of brain-derived neurotrophic factor (*Bdnf*) in hippocampus of mice exposed to environmental enrichment or chronic stress are associated with opposite changes in the H3K27me3 mark at a subset of *Bdnf* gene promoters [12,57]. In addition, acute stress leads to an overall decrease in hippocampal H3K27me3 and H3K9me3 [58]. Furthermore, in the orbitofrontal cortex of suicide completers, alterations in H3K27 methylation were described at the *TRKB* gene, encoding the high affinity receptor for the nerve growth factor molecule, BDNF [27]. Changes in the balance between histone H3K4 and H3K27 methylation, or DNA cytosine and H3K27 methylation may also contribute to GABAergic gene expression deficits in schizophrenia [28,43]. To date it remains unclear which of the various H3K27-specific KMTs and KDMs (Fig. 1) are involved in these disease-related alterations in postmortem brain tissue. Of note, the Jumonji and Arid containing protein 2 (JARID2), which by itself lacks catalytic activity but is crucial for subsequent H3K27 or H3K9 methylation by recruiting the polycomb PRC2 complex to its target genes [59,60], is located within the schizophrenia susceptibility locus on chromosome 6p22 and confers genetic risk in multiple populations of different ethnic origin [61,62]. While the biological functions of JARID2 have been studied primarily in the context of transcriptional regulation in stem cells [63,64], this gene shows widespread expression in the mature nervous system [65], implying JARID2-mediated control over polycomb repressive chromatin remodeling in the adult brain.

H3K36 and H4K20

Epigenetic dysregulation of nuclear receptor-binding SET domain containing protein 1/KMT3B could play a role in some neuro- and glioblastomas [66], but like for other H3K36 and H4K20 regulating enzymes (Fig. 1), to date little is known about their role in neurodevelopment, cognition and psychiatric disease. Strikingly, however, KMT3A/HYPB/SETD2, a member of the SET2 family of KMTs mediating H3K36 methylation [67], is also known as huntingtin-interacting protein 1 (HIP-1) or huntingtin(yeast)-interacting protein B (HYPB) [68]. Huntington's is a triplet repeat disorder and chronic neurodegenerative condition with motor symptoms and cognitive defects, and significant changes in mood and

affect [69]. Whether or not there is altered H3K36 methylation in the neuronal populations that are at risk for degeneration is unclear. Furthermore, the huntingtin/KMT3A interaction has been documented for yeast [68] but not brain. Of note, wildtype huntingtin is a facilitator of polycomb complex PCR2-mediated H3K27 methylation [70], and furthermore, H3K4 and H3K9 methylation changes have been reported in preclinical model systems and postmortem brains with Huntington's disease [71,72]. Therefore, it is possible that transcriptional dysregulation in this condition is associated with aberrant methylation patterns of multiple lysine residues.

KMTs and KDMs as Novel Drug Targets

Given the emerging role of histone methylation in the neurobiology of psychiatric disease, the next obvious question is whether this type of PTM could provide a target for a new generation of psychotropic therapeutics. In principle, KMTs and KDMs should provide fertile ground for the development of novel drugs, because these enzymes are considered more specific than, for example, HDACs, because each HDAC enzyme is likely to affect a much larger number of histone residues as compared to KMTs/KDMs [73]. However, like for other histone modifying enzymes, the specificity of KMTs and KDMs is not limited to histones but includes the (de)methylation of lysines of non-histone proteins, including the p53 tumor suppressor protein and the VEGF growth factor [74]. Druggable domains within the KMTs and KDMs could involve not only their catalytic sites, such as the SET domain for the KMTs or the amino oxidase and JmjC domains for the LSD1 and JMJD subtypes of KDMs, respectively, but also some of the many other functional domains that are specific to subsets of these proteins [75]. One potential candidate would be the bromodomain of the MLLs and other H3K4-specific methyltransferases [75]. Bromodomains, which are present in many different types of nuclear proteins, bind to acetylated histones and small molecules interfering with some of these interactions recently emerged as powerful modulators of systemic inflammation [76].

The catalytic activity of the SET domain containing KMTs requires the universal methyl donor, S-adenosyl-methionine (also known as AdoMET). Crystallographic and functional studies revealed that the SAM binding pocket of KMTs is different from the SAM pockets of other proteins, which may increase the chance to develop compounds which specifically target histone methyltransferases but not other enzymes and proteins [31]. Currently, however, no KMT or KDM related drug is in clinical trials. However, several of these compounds show therapeutic promise in preclinical studies. For example, the S-adenosylhomocysteine hydrolase inhibitor, 3-deazaneplanocin A (DZNep) induces apoptosis in breast cancer cells [77]. This drug alters H3K27 and H4K20 trimethylation via interference with polycomb PRC2 repressive chromatin remodeling [73]. Antioncogenic effects were also observed with BIX-01294, a drug that downregulates H3K9 methylation levels by binding to the SET domain of the G9a/GLP(EHMT1) methyltransferases [73]. The same drug was shown to alter addictive behaviors and H3K9 methylation when infused locally into the brain of cocaine-exposed mice [48]. As discussed above, while tranylcypromine and other monoamine oxidase inhibitors used for the treatment of depression are weak inhibitors of the LSD1 type of KDM, recently several compounds emerged with much stronger activity against LSD1/LSD2 [18]. It will be extremely interesting to explore these drugs in preclinical models for mood and psychosis spectrum disorders. Finally, microRNA-based therapeutic strategies, aimed at decreasing levels and expression of chromatin remodeling complexes, including some of the histone modifying enzymes discussed here, are gaining increasing prominence in the field of cancer therapy [73] and may in the future emerge as a novel therapeutic option in the context of neuropsychiatric disease.

Synopsis and Outlook

Alterations in histone methylation, both on the level of global ('bulk') chromatin and more specifically at select genes and loci, were reported in postmortem brain of subjects diagnosed with schizophrenia or depression [27,28,43,78]. Histone methylation serves as a complex epigenetic regulator in the brain and other tissues, with multiple sets of residue-specific methyltransferases and demethylases, further diversified in terms of the composition and structures of their catalytic and other functional domains. Genetic studies in mouse and human implicate multiple lysine methyltransferases and demethylases, including MLL1, G9a/GLP(EHMT1), SETDB1, SMCX/JARID1c and a related gene JARID2, in the regulation of cognition and motivational and affective behaviors and could in the near future become a therapeutic target for a variety of psychiatric conditions. With an estimated 100 lysine and arginine residue-specific histone methyltransferases and demethylases encoded in the genome, these epigenetic enzymes are expected to provide plenty of targets for drug discovery [31].

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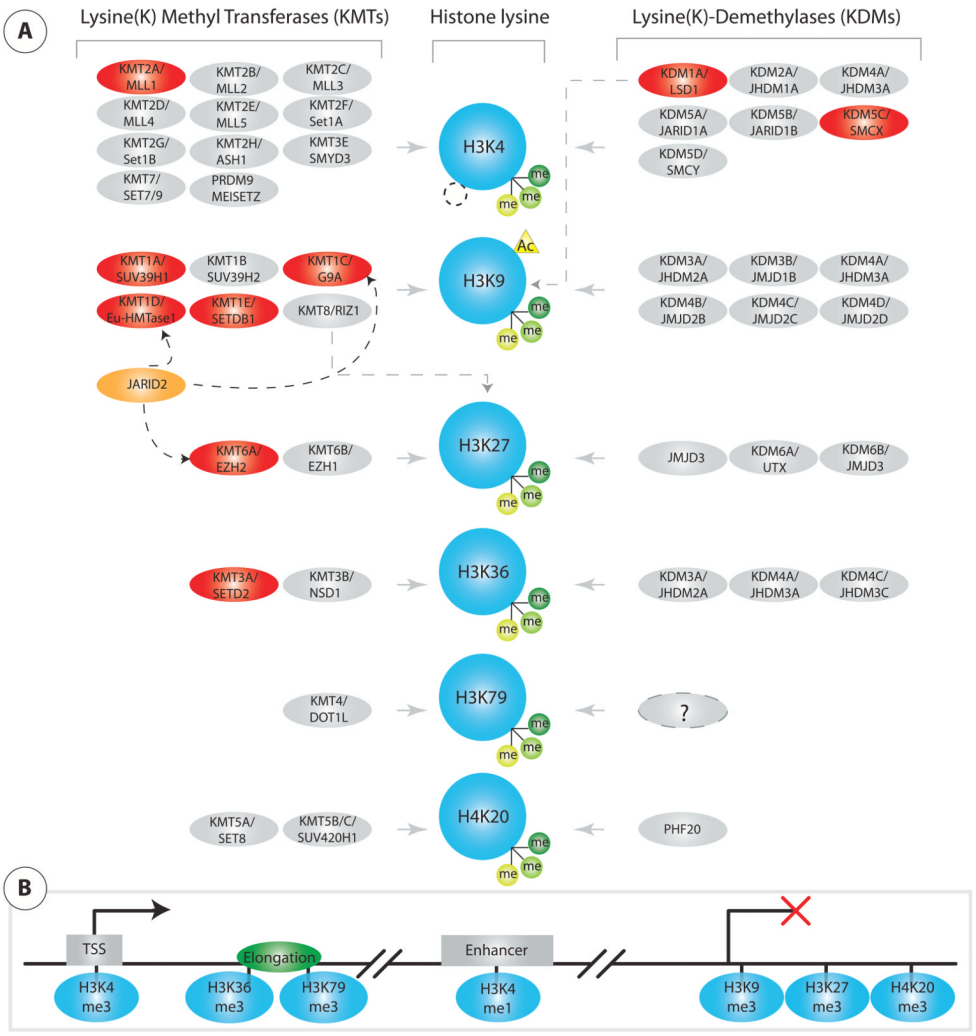

Figure 1.

Regulation of histone (K) methylation. **(A)** Listings of residue-specific KMTs and KDMs for H3K4/9/27/36/79 and H4K20. The majority of KMT and KDM are highly specific for a single histone residue, while a few enzymes target multiple residues, as indicated. Red marked KMT/KDM are implicated in neurodevelopment or psychiatric disease as discussed in main text. The non-catalytic JARID2 regulates activity and function of related KMTs. (**B**) Simplified scheme for selected mono- and trimethylated histone lysine markings implicated in transcriptional regulation, silencing and enhancer functions.

Table 1

Drug-induced histone methylation changes

Drug(s)	Type	Histone K residue(s)	Genes epigenetically altered in brain	Comments
clozapine	Atypical antipsychotic with broad affinity for dopamine D2, serotonin, muscarinic and other receptors	*Trimethyl-H3K4*	*GAD1* GABA synthesis enzyme [28]	increased *GAD1*-H3K4 methylation after clozapine exposure both in mouse and human prefrontal cortex [28]
valproate, butyrate	Inhibitors of class I/II histone deacetylase (HDACs); valproate is a mood-stabilizer and anticonvulsant	*Trimethyl-H3K4*	*Gad1* and other GABAergic gene promoters [28]	Fear conditioning and butyrate treatment in mice induces global increase in hippocampal H3K4 methylation [36]
phenelzine, tranylcypromine	Antidepressants inhibiting monoamine oxidases MAO-A/B	*Di-, Mono-methylH3K4/9*	unknown	Direct inhibitors of LSD1 histone demethylase (amine oxidase superfamily)[79]
cocaine	Stimulant drug-of-abuse and monoamine reuptake inhibitor	*DimethylH3K9 Trimethyl-H3K4, Trimethyl-H3K27*	Cdk5, NFkB, Bdnf, Arc, FosB and others [48]	Cocaine induces downregulation of G9a/GLP H3K9 methyltransferase in ventral striatum [48] and global H3K4 and H3K27 demethylation in prefrontal cortex [80]