Epigenomics

New hope: the emerging role of 5-hydroxymethylcytosine in mental health and disease

Historically biomedical research has examined genetic influences on mental health but these approaches have been limited, likely due to the broad heritability of brain-related disorders (e.g., 30–90%). Epigenetic modifications, such as DNA methylation, are environmentally sensitive mechanisms that may play a role in the origins and progression of mental illness. Recently, genome-wide disruptions of 5-hydroxymethylcytosine (5hmC) were associated with the development of early and late onset mental illnesses such as autism and Alzheimer's disease, bringing new hope to the field of psychiatry. Here, we review the recent links of 5hmC to mental illness and discuss several putative functions of 5hmC in the context of its promising clinical relevance.

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A fundamental problem in psychiatry is that diagnoses are based on subjective measures that are primarily acquired through behavioral assessments. Modern genetics has held out hope that one could identify new genetic markers of mental illness early in childhood that could aid the subjective measures of behavior used today. New laboratory tests for these markers would then indicate to psychiatrists the most promising drug for each person or direct pharmaceutical companies to more specific personalized drug development. However, even though complete mapping of the human genome was announced in 2003, the genetic approach has had little impact on improving the lives of individuals struggling with mental illness. This outcome is partially caused by the broad range of heritability in mental illness (i.e., 30–90%) but also is due to a failure in molecular psychiatry that has overlooked a crucial molecular step that causes variations in the expression of key genes in the brain. Past approaches have largely ignored how environmental stimuli can affect the unique amount of gene expression in the brain of each person. Thus, the use of this personalized information provides new hope that one day it will be possible to develop a precise molecular-based treatment, which will change the way mental health is diagnosed and treated today.

Epigenetic mechanisms are environmentally sensitive molecular modifications that are heritable but do not change the underlying DNA sequence. Much effort has been put forth toward understanding two specific epigenetic mechanisms: histone modifications, which alter gene expression through the restructuring of chromatin proteins, and DNA methylation, which modulate gene expression through the addition of a methyl group to specific sites within the DNA sequence. The most studied epigenetic mechanism in the mammalian genome is DNA methylation, which is the addition of a methyl group on the fifth carbon of cytosine (e.g., 5-methylcytosine [5mC]). This DNA mark functions in genomic imprinting,

X-chromosome inactivation, chromatin structure and gene silencing [1–5]. For a more definitive and technical perspective of 5mC, please refer to Box 1. Human studies also support a role for 5mC in the development of mental pathologies including bipolar disorder, schizophrenia and major depressive disorder, often resulting in concomitant changes in gene expression [6–10]. It was recently shown that 5mC can be oxidized to 5-hydroxymethylcytosine (5hmC) and that this modification is environmental sensitive [11], stable [12] and highly enriched in the brain [13–17]. While the molecular role(s) of 5hmC remain elusive, primarily due to the dearth of 5hmC functional studies, its unique enrichment in the brain [18] leads many to believe that 5hmC functions are independent from those of 5mC, and that 5hmC may be a critical molecular building block in mental health that is disrupted in disease. For a more definitive and technical perspective of 5hmC, please refer to Box 1. It is noteworthy that traditional DNA methylation detection methods utilizing sodium bisulfite treatment cannot distinguish between the methylated and hydroxymethylated forms of cytosine, meaning that past studies that utilized such methods report a composite of 5mC and 5hmC but have attributed any findings solely to 5mC. This review will provide substantial evidence to support the independent importance of 5hmC in mental health and the development of mental illness and present several putative

molecular functions of 5hmC that may shed light on its promising clinical relevance. With recent findings implicating a unique role for 5hmC in developmental brain disorders, 5hmC brings a new frontier to the field of psychiatry.

Development, aging & neurodegeneration

5hmC is essential for proper mammalian development, beginning with an early role in the distinction between embryonic stem cells (ESCs) and neural progenitor cells (NPCs) [33]. Here, the transition from ESC to NPC requires a genome-wide reduction of 5hmC, which is facilitated by a dramatic change in the expression of the modulatory proteins required for oxidizing 5mC to 5hmC (TET1-3; Box 1) [34]. These findings link 5hmC and TET expression with ESC differentiation and suggest that aberrant 5hmC levels may impact neurogenesis. Consistent with this link, embryos completely lacking TET1 and TET3 experience lower 5hmC and higher 5mC levels, which results in more variable expression of important neurodevelopmental genes including those in the cholesterol biosynthesis pathway that lead to the development of holoprosencephaly, a cephalic disorder characterized by the failure of the two brain hemispheres to properly form [35]. To our knowledge, this is the only study that has been able to link deficits of 5hmC with phenotypic alterations in embryos. While these studies demonstrate a role

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Box 1. DNA methylation.

5mC

- 5mC is a modified version of cytosine that involves the addition of a methyl group to the fifth carbon of cytosine and is nearly exclusive at CpG dinucleotide sites. This modification is catalyzed by DNMTs, which utilize S-adenosyl-L-methionine as the methyl donor [19]. Three active DNMTs have been identified in mammals and they have distinct functions. DNMT3a and DNMT3b have an affinity toward unmethylated CpG sites, while DNMT1 preserves methylation, as it shows preference toward hemimethylated CpG sites [20]. While CpG rich-regions, known as CpG islands, and gene promoters have a significant reduction in 5mC levels, the X chromosome has an overabundance of 5mC [21,22]. 5mC regulates processes such as gene silencing, X-chromosome inactivation, genomic imprinting and chromatin structure [23]. The 'gold-standard' in the detection of 5mC is sodium bisulfite sequencing, which converts unmethylated cytosine to uracil that are read as thymine following PCR [24]

5hmC

- 5hmC is a modified version of cytosine that involves the addition of a hydroxy group to the methyl group of 5mC. This modification is catalyzed by the TET family of enzymes [25]. An approximately tenfold abundance of 5hmC is seen in brain tissues, other tissues of the central nervous system and embryonic stem cells, compared with peripheral tissue [26]. 5hmC levels are reduced in CpG islands and intronic sequences, but are enriched in gene promoters, the 5'-UTR, exons, and distal regulatory regions [26]. Notably, mammals have a disproportionate reduction of 5hmC on chromosome 18 and the X chromosome [27]. While an increase in transcription levels has been correlated with 5hmC enrichment [26], alternatively spliced transcripts display a reduced 5hmC on excised exons as compared with constitutive exons, suggesting that 5hmC may be playing a role in the production of isoform transcripts [28]. Neuronal- and synapse-related genes have a significant increase in 5hmC levels [28]. The function of 5hmC has yet to be discovered, yet several putative functions are discussed in this review, including the regulation of transcription factor binding [29], gender-specific development [30] and transcript diversity through interactions with the spliceosome [31]. Since traditional sodium bisulfite sequencing cannot distinguish between 5hmC and 5mC, Tet1-assisted bisulfite sequencing has become an established method for 5hmC detection [32]

5hmC: 5-hydroxymethylcytosine; 5mc: 5-methylcytosine.

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for 5hmC in neurodevelopment during embryogenesis, 5hmC continues to be abundant postnatally and undergoes age-dependent accumulation throughout early life development and into adulthood [36–38]. However, environmental stimuli, such as caloric restriction, can alter this age-associated accumulation of 5hmC, as caloric restricted mice showed an age-dependent reduction of 5hmC in hippocampal and cerebellar tissue [39]. Together, these findings suggest that neurodevelopmental age and environment strongly influence 5hmC dynamics in the brain, which in turn can play a modulatory role in the expression of key genes involved in neurodevelopment.

The emerging link between 5hmC and brain development, and its accumulation with age, have led researchers to examine this epigenetic mark in neurodegenerative diseases [40]. Indeed, age-associated genes that acquire 5hmC are associated with pathways related to neurodegenerative diseases [25]. For example, depletion of 5hmC was found in the hippocampus, cerebellum and entorhinal cortex of patients suffering from Alzheimer's disease (AD) [41,42], a type of dementia that usually develops slowly and gets worse over time. On the other hand, enrichment of 5hmC in the frontal and midtemporal gyrus was positively correlated with hallmarks of AD, including neurofibrillary tangles (NFT), amyloid beta and ubiquitin load [43]. Together, these findings suggest that 5hmC may be driving the primary molecular components of AD progression within distinct regions of the brain. Notably, AD-associated levels of 5hmC can be detected at preclinical stages as well as at later stages of AD [44], indicating that 5hmC may act as a viable biomarker of AD onset and progression. However, studies employing nuclear labeling or ELISA were unable to find significant alterations in 5hmC associated with AD [45], suggesting that higher resolution methods are required to identify AD-associated changes in 5hmC. Connections between 5hmC and other neurodegenerative disorders including Huntington's, ataxia-telangiectasia and Fragile X-associated tremor/ataxia syndrome have recently surfaced and we will describe the link to 5hmC of each of these disorders below.

Huntington's disease (HD) is characterized by deficits in cognitive, psychiatric and motor stability that is typically caused by a CAG trinucleotide expansion in the *HTT* gene [46]. In addition to the mutation in the *HTT* gene, previous studies have suggested a role for epigenetic mechanisms in HD, such as altered histone modifications [47] and DNA methylation of a locus closely linked to the *HTT* gene known as DS495 [48]. These epigenetic connections led to the recent investigation of the genome-wide distribution of 5hmC in a mouse model of HD, which found a deficiency of 5hmC in the mouse striatum and cortex tissues [49]. This altered distribution of 5hmC was generally associated with pathways involved in neuronal development/differentiation, axonal guidance and neuronal function/survival. A specific example included a 5hmC reduction on the *ADORA2A* gene, a G-protein-coupled receptor that decreases its expression in HD patients, which revealed that 5hmC may play a role in the pathological expression of *ADORA2A* found in HD patients [50]. Together, these results implicate the loss of 5hmC as a potential molecular mechanism underlying the cause of neuronal cell death associated with HD.

Ataxia-telangiectasia, a devastating neurodegenerative disorder that shows high cell specificity in its development, is caused by a mutation in the *ATM* gene [51]. Despite this monogenic etiology, alterations in 5hmC were recently found in repeated sequences and regulatory elements of DNA from *ATM*-deficient Purkinje cells from mice and humans [52]. This 5hmC distribution is likely the result of TET1 responding to DNA damage, which is a hallmark of *ATM*-deficient neuronal cells. To test whether TET1 activity is related to Purkinje cell degeneration and behavioral deficits in mice, adenoviral particles encoding either human wild-type TET1 (TET1-WT) or kinase dead mutant TET1 (TET-KD) were infected into wild-type and *ATM*-deficient mouse cerebellum slices. Cerebellum samples lacking TET1 expression that were infected with TET1-WT showed no activation of *CASPASE-3*, a cell death marker, while infection with TET1-KD activated *CASPASE-3*. These findings suggest that TET1-mediated 5hmC production is essential for Purkinje cell viability and the prevention of ataxia-telangiectasia-like symptoms in mice, supporting the concept that vulnerability to neurodegeneration is linked to aberrant changes of 5hmC in neuronal cells.

Fragile X-associated tremor/ataxia syndrome (FXTAS), a late-onset neurodegenerative disorder, is one of the characterized Fragile-X disorders that are caused by a CGG expansion in the 5'UTR of the *FMR1* gene [53,54]. Genome-wide analysis of 5hmC in the cerebellum of a FXTAS mouse model (rCGG mice) revealed an overall reduction in 5hmC at 16 weeks of age when compared with age-matched controls [55]. Despite the overall reduction of 5hmC, these mice have an increase of 5hmC in repetitive sequences as well as in cerebellum-specific enhancers. Differential 5hmC between the rCGG and control mice were predominantly found in transcription factor (TF) binding sites that are located in genes essential for neuronal development. Finally, ribosomal profiling revealed that the differential 5hmC-associated genes often exhibited altered ribosomal processing in the rCGG mice,

suggesting that 5hmC may somehow influence translational changes. In summary, this study links 5hmC to the etiology of FXTAS and implicates a role for 5hmC in TF binding and in regulating ribosomal processing of mature RNA transcripts.

Despite that the above disease associated disruptions in 5hmC represent changes across age; these examples clearly demonstrate a role for 5hmC in proper development and in the onset and progression of neurodegenerative diseases. These developmental and neurodegenerative disease-associated changes in 5hmC often arise within distinct cell-types and brain regions, supporting cell and tissue-specific development of these diseases. Since these studies have been largely descriptive, it will be imperative to determine the functional mechanism(s) played by 5hmC if we are to modify it toward healthy outcomes.

Sensitivity to environmental stimuli

The abundance and distribution of 5hmC is sensitive to environmental stimuli. Mice exposed to enriched environment show reduced 5hmC abundance in the hippocampus primarily on genes involved in axon guidance. These alterations were also associated with increased learning and memory, suggesting that environmental enrichment might modulate the dynamics of 5hmC in the hippocampus and contribute to improved learning and memory [56]. In contrast, our group found increased 5hmC abundance on the glucocorticoid receptor gene (*Nr3c1*) in the hippocampus of mice stressed for 30 min and allowed to recover for 1 h [57]. Genome-wide 5hmC analysis of these same mice revealed that short-term stress induced genome-wide disruptions of 5hmC and confirmed an overall increase in 5hmC following stress. Similar to the FXTAS mouse model described above, altered 5hmC was found in several TF binding sites of genes. Here these genes also were differentially expressed and have known roles in neurogenesis and neurological activities [29], suggesting that in response to stress the function of 5hmC may be to influence TF binding to provide appropriate levels of gene expression needed to cope with the stress. The fact that 5hmC changes were found within 1 h following a short stress highlights the potential for rapid changes of 5hmC within the brain. It will be interesting to examine the long-term effects of short stress (i.e., more than 1 h after exposure) or how chronic stress alters 5hmC levels. Many believe 5hmC to be important in long-term consequences of mental health, yet this study indicates that alterations in 5hmC can occur rapidly and may impact the expression of key genes related to the origins of mental illnesses. While environmental stressors have been shown to alter gene expression [58], a definitive molecular culprit has been slow to emerge, perhaps 5hmC is a promising candidate.

Environmental stimuli affects brain regions other than the hippocampus. For example, mice exposed to repeated administrations of cocaine have increased 5hmC in the nucleus accumbens, primarily in coding regions and enhancer sequences of genes involved in drug addiction [31]. Notably, 5hmC changes persisted for a minimum of 1 month after cocaine exposure in only a small subset of loci, suggesting that these epigenetic changes are largely reversible. In addition, modulation of 5hmC may also mediate behavioral adaptations. For example, fear extinction, a form of reverse learning, results in dramatic 5hmC changes in the prefrontal cortex of mice [59]. These studies also supported unique molecular roles for the TET enzymes, as TET3, but not TET1 mediated the increased gene expression that was associated with rapid behavioral adaptation. Interestingly, another group found that mice lacking the expression of *Tet1* exhibited impaired memory extinction, coupled with long-term synaptic depression and down-regulation of neuronal activity-related genes [60]. Thus, while TET3 may solely facilitate the accumulation of 5hmC in the prefrontal cortex of mice during rapid behavior adaptation in response to fear, TET1 governs alterations in 5hmC on synaptic plasticity genes during behavioral adaptation in response to stressful environmental exposures.

Taken together, these studies open up the possibility that 5hmC may function in the development of environmentally sensitive neuronal dysfunction. It will be of great interest to investigate the role of each TET enzyme coupled with the rapid and stable dynamics of 5hmC at different developmental time points to understand its role in synaptic plasticity, neuronal development, the maintenance of mental health and the onset of mental illness.

Early life adversity, 5hmC & the origins of psychiatric disorders

Several studies indicate that early life experiences have a profound impact on brain development and subsequent adult behavior [61–64]. Recent evidence indicates that the epigenome is a potential molecular mechanism governing the long-lasting effects of early life stress on brain and behavior. One such example involves rhesus macaques that were deprived of early life maternal interactions. As adults, these monkeys have altered 5hmC in the prefrontal cortex on promoters of genes related to neurological functions and psychological disorders (e.g., *DRD3*, *5-HTT*, and *GABRA2*) [65]. Since these 5hmC disruptions were detected during adulthood, early life changes in 5hmC also can be stable throughout development and may represent

the origins of developmental brain disorders such as schizophrenia, bipolar disorder and autism.

Schizophrenia, bipolar disorder & major depressive disorder

Schizophrenia (SCZ) and bipolar disorder (BD) are psychiatric disorders with shared and distinct clinical and genetic features. In both disorders, stressful events increase the risk for onset and relapse mainly through the dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis. Although many genes are linked to HPA dysfunction, the majority of SCZ and BD cases cannot be explained by genetics alone and the epigenome likely has a role in the etiology of these disorders. Consistent with this hypothesis, rodent models exposed to prenatal stress exhibit long-lasting neurological, endocrinological and behavioral changes that are thought to mirror the development of SCZ [66]. These models show increased DNA methylation (5mC + 5hmC) in GABAergic interneurons primarily in CpG-rich regions of GABAergic genes [67,68]. Together, these studies demonstrated that prenatal stressors can alter DNA methylation levels in the brain and implicate a role for DNA methylation in the origins of SCZ and mood disorders [69]. In humans, an independent role for 5hmC in SCZ and BD patients is characterized by increased 5hmC abundance and TET1 expression, but not TET2 or TET3, in the inferior parietal lobule (IPL) [70]. Remarkably, TET1 was not altered in the cerebellum of these patients, suggesting that 5hmC may be involved in the development of psychosis through the inferior parietal lobule, but not the cerebellum, perhaps shedding light on the tissue-specific development of SCZ and BD. The increases of 5hmC in these patients were associated with reduced expression of biologically relevant genes including *GAD67* and *APOBEC3A*, which is an enzyme with critical roles in the active DNA demethylation pathway. In contrast, patients only suffering from severe major depressive disorder have significantly decreased levels of 5hmC [71], suggesting that 5hmC has unique functions among closely related brain disorders. Together, these findings suggest a common etiology in psychosis, one that includes early life adversity and genome-wide changes in 5hmC.

Autism

The autism spectrum disorders (ASD) encompass a broad range of behaviorally related and neurodevelopmental disorders with a high prevalence in children. Notably, only ~20% of ASD cases show a genetic etiology [72,73]. Prenatal factors shown to increase the risk of ASD in offspring [74] include environmental influences such as multiple births, *in vitro* fertilization and parental exposure to common drug treatments (e.g., antiepileptic drugs [e.g., valporate] or folic acid) [75]. Together, these findings effectively open the door for contributions from epigenetic modifications such as 5hmC to have an underlying role in the ASD etiology. Consistent with this hypothesis, developmentally specific changes in 5hmC are highly enriched in known autism genes [76]. Moreover, we recently profiled 5hmC in a mouse model of autism that has behavioral and molecular alterations such as deficits in social interactions and GABAergic signaling [77,78]. This study revealed that this mouse model harbored differential 5hmC on a remarkable number of established human autism genes, suggesting that 5hmC may be influencing the observed autistic-like phenotype in these mice [79]. Since these findings were observed post-symptomatically in adult mice, it is unclear if the altered 5hmC represents a cause or a consequence of having autistic-like behaviors. Thus, these findings warrant a deeper investigation of this mouse model at earlier developmental time points.

Early life stressors have been associated with the development of mental illness, but the extent to which a gene x environment interaction contributes to the development of autism has yet to be tested. However, prenatal stress reduced anxiety-like behaviors and altered gene expression in mice heterozygous for the *5-HTT* gene [80]. Interestingly, these findings were more pronounced in female offspring, suggesting a gender-specific development of these altered behaviors. This study demonstrates a gene × environment interaction and while this study did not examine 5hmC, the 5hmC-related data discussed in this review supports a 5hmC contribution to these altered behaviors and gene expression. Thus, it will be interesting to examine whether early life stress causes an autism-prone individual (e.g., heterozygote for an autism risk allele) to develop full-blown autism phenotype, and if 5hmC plays a role in this process. If so, do these changes in 5hmC mirror those found in the homozygous mutants. This type of study could show that early life stress affects outcomes in offspring that may have a genetic predisposition to autism, a likely scenario for many mental disorders.

Putative functions/clinical utilities of 5hmC

While many studies have shown that disruptions in 5hmC are linked with mental illness, the precise molecular function(s) of 5hmC remains unknown. Since 5hmC is generally associated with the transcription of neuronal genes, it likely has a role in the regulation of gene expression. Since 5hmC is enriched in post-mitotic neurons, it most likely has an impact on proper neurodevelopment by regulating expres-

sion of genes responsible for neuronal propagation, development, and maintenance.

Several studies have revealed that altered 5hmC in differentially expressed genes is proximal to TF binding motifs [29,55]. Therefore, 5hmC may regulate TF binding to DNA, resulting in either up- or down-regulation of gene expression (Figure 1). 5hmC also has been associated with the regulation of alternative splicing of transcripts after cocaine-administration [31]. Here there was an overall greater change in isoform transcript levels, compared with full-length transcript levels [81]. An increase in 5hmC also was found in splice sites associated with upregulated spliced isoforms and a decrease of 5hmC in splice sites associated with downregulated spliced isoforms; hence, again coupling 5hmC changes in the brain with alternative splicing (Figure 1) [31]. Together, these studies implicate 5hmC in the regulation of transcriptional abundance and diversity.

5hmC may also affect the binding of miRNAs (miRs). We recently discovered that altered 5hmC in differentially expressed genes is proximal to sequences that are complementary to seed regions of several miRs [Madrid, Alisch, Unpublished Data]. While miRs are generally thought to bind to mRNA and not DNA, 5hmC may have a co-transcriptional function in attracting or repelling miRs as the DNA is being transcribed into RNA (Figure 1). Interestingly, chronic variable stress on male mice alters miR content in sperm, and offspring from these sperm have a reduction in HPA axis stress responsivity [82]. Thus, miRs and 5hmC may work together in response to early life stressors by altering gene expression and physiological sensitivity to adversities, which may contribute to the origins of mental illnesses.

The instability of 5hmC levels following prenatal and/or acute stress underscores the potential for 5hmC
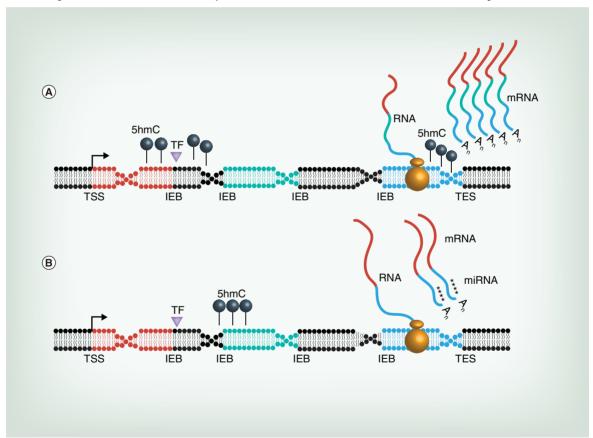

Figure 1. Schematic of putative functions of 5hmC. When located near TF binding motifs (purple triangle), 5hmC (black lollipops) may regulate gene expression levels, resulting in either up- **(A)** or down- **(B)** regulation. When located at IEB, 5hmC (black lollipops) may regulate alternative splicing of transcripts, resulting in a shift from full-length transcripts **(A)** to splice isoforms **(B)**. Exons are colored red, green, and blue. Intergenic and intragenic regions are gray. When located near sequences complementary to miRNAs seed regions, 5hmC (black lollipops) may have a latent function in repelling **(A)** or attracting **(B)** miRNAs as the DNA is being transcribed into RNA. Group of four asterisks represent miRs. Together, these studies implicate 5hmC in the regulation of transcriptional and translational abundance and diversity.
5hmC: 5-hydroxymethylcytosine; IEB: Intron/exon boundaries; TES: Transcription end site; TF: Transcription factor; TSS: Transcription start site.

as a novel biomarker in the diagnosis of mental health. In addition, the presence/absence of these epigenetic modifications is reversible; thus, they may become relevant in therapeutic interventions [83], especially if methods to selectively modulate 5hmC *in vivo* are developed at the nucleotide level. Finally, 5hmC has gender-specific profiles, which is of interest because the development of several psychiatric disorders are seemingly gender-specific. For example, females show an increased risk in developing anxiety and depression while males show a disposition to development of autism and attention deficit hyperactivity disorder [84,85]. A recent study found gender-specific 5hmC on genes with ontological terms correlating with organ morphogenesis, system development and development of anatomical structures [30], suggesting that 5hmC may differentially influence the development of organs (e.g., the brain) among the genders. Together, these factors must be considered for clinical application and treatment endeavors.

In reality, our understanding of 5hmC functions is obscure and in its infancy. These molecular roles are likely to contribute to a broad spectrum of cellular functions from lifelong neurogenesis to cell death. Environmentally sensitive molecular mechanisms, such as 5hmC, in the brain have become a significant focus of neuroscience research because of growing evidence that they are critical to the development of psychiatric disorders. Thus, in the coming years it will be of great interest to unravel these molecular functions of 5hmC that are contributing to developmental brain disorders.

Conclusion

Many of the studies discussed here suggest that mental illness is associated with disruptions in 5hmC throughout the genome. Since 5hmC is present embryonically and accumulates throughout life, primarily on genes associated with development, these studies support a role for 5hmC in developmental brain disorders. In addition, finding that 5hmC levels also can rapidly change at certain loci to reflect environmental stimuli and that these changes also can become stable, opens new perspectives in the study of epigenetic mechanisms underlying mental health and the origins of mental illness. The growing number of links between 5hmC and psychiatry disorders leads many to suspect a functional role for 5hmC in mental health. While it is still unknown whether these mental illness-related disruptions in 5hmC are causative or correlative to the outcome, defining the molecular function(s) of 5hmC will likely reveal the clinical relevance and should lead to the development of new, more specific drug therapies with more personalized therapeutic options for

those with mental illness. Together, these studies highlight the emerging role of 5hmC in mental health and disease and bring new hope to the field of psychiatry.

Future perspective

This review has highlighted the new hope that the study of 5hmC brings to psychiatry. While traditional molecular genetics has been limited in revealing the etiology of mental illness, 5hmC may provide a viable molecular mechanism contributing to the onset and progression of such disorders and diseases. A more comprehensive understanding of 5hmC, both functionally and as a biomarker, may provide a more objective measurement toward a more precise diagnosis of patients with mental illness. Ultimately, the development of methods to modulate 5hmC at the nucleotide level in genes contributing to mental illness may direct pharmaceutical companies in more fruitful directions of drug development for the treatment of mental illness. One such approach that might help achieve this nucleotide-specific modulation of 5hmC within the genome is to tether clustered, regularly interspaced short palindromic repeats (CRISPR) segments of DNA with either DNA methyltransferases or TET enzymes to selectively methylate or hydroxymethylate specific regions of the genome, respectively. Such a method would allow for specific modulation of 5hmC on genes that are known to have altered of 5hmC in mental illnesses, potentially allowing for personalized treatment and prevention of psychiatric disorders.

5hmC in the context of mental illness is new and rapidly growing, suggesting that the future of such research is bright and prospectively fruitful. Our group expects that in the coming years disruptions of 5hmC will be examined in a cell-specific fashion, allowing researchers to study 5hmC at a much higher resolution. Future cell-specific examinations of genome-wide 5hmC in specific neural circuits that contribute to behavioral outcomes will provide an objective marker for a much quicker more reliable diagnosis that will lead to personalized treatments for those at high risk of developing a full-blown mental disorder.

Executive summary

State of psychiatry
- Diagnosis is based on subjective measures that are primarily acquired through behavioral assessments.
- Genetic influences often sought to explain the etiology of mental health.
- The broad heritability and heterogeneity of mental illness has limited the success of molecular genetics to determine its etiology.
- Crucial molecular mechanisms that cause variations in the expression of key genes in the brain are currently ignored by molecular psychiatry, which is contributing to misdiagnoses and ineffective treatments: a fundamental problem in the field of psychiatry.

Epigenetics & 5-hydroxymethylcytosine
- Environmentally sensitive modifications that are heritable and often induce stable changes in gene expression.
- 5-hydroxymethylcytosine (5hmC) is a recently identified modification of cytosine that shows significant enrichment in brain over peripheral tissues and on genes known to have neuronal and synaptic functions.
- 5hmC is present embryonically and accumulates throughout life, suggesting a potential lifelong role in development.
- The presence/absence of 5hmC modifications are reversible.

5hmC in mental health
- 5hmC disruptions have been associated with the development of early and late onset mental illnesses such as autism and Alzheimer's disease.
- Although linked to mental health, disruptions of 5hmC are not currently known to be causative or correlative of mental stability.
- Function(s) of 5hmC remain elusive, warranting deeper investigations aimed at unlocking the full potential of 5hmC as a therapeutic measure of mental health.
- Potential biomarker in the personalized diagnosis of mental illness.

Potential functions of 5hmC
- Current understanding of 5hmC function(s) is obscure and in its infancy.
- Alterations of gene expression may be through transcription factor binding, miRNA binding and/or alternative splicing of transcripts to generate isoforms.
- Gender-specific distributions, which may explain gender-specific dispositions to mental illness (e.g., depression in females and autism in males).

Future perspective
- The development of personalized therapeutic manipulations of 5hmC to prevent onset or progression of mental illness through methods such as CRISPR to target specific genomic loci.
- The development of cell-specific profiling of specific neural circuits that contribute to behavioral outcomes will provide an objective marker for a much quicker and more reliable diagnosis that will lead to personalized treatments for those at high risk of developing a full-blown mental illness.
- The study of 5hmC brings new hope to the field of psychiatry through its promising clinical relevance.

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