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The Molecular Pathology of Schizophrenia – Focus on Histone and DNA Modifications

Schahram Akbarian
Brudnick Neuropsychiatric Research Institute, Department of Psychiatry, University of Massachusetts Medical School, Worcester, MA 01604, Phone 508 8562674, Fax 508 8562627

Schahram Akbarian: Schahram.akbarian@umassmed.edu

Abstract

Dysfunction of cerebral cortex and other brain regions in schizophrenia is often accompanied by dysregulated expression of numerous genes. However, the underlying genetic risk architecture remains unclear for a large majority of cases. Therefore, the study of epigenetic regulators of gene expression, including covalent modifications of DNA and nucleosome core histones, offers an attractive alternative to further explore the molecular pathology of schizophrenia beyond the level of RNA quantification. Several studies reported alterations in DNA cytosine methylation and histone methylation at specific genes and promoters in postmortem brain of subjects with schizophrenia, often in conjunction with changes in levels of the corresponding RNAs. While evidence for such “epigenetic dysregulation” is increasing, many of the reported alterations await independent replication. Interestingly, studies across the lifespan indicate that DNA and histone methylation markings are developmentally regulated in human cerebral cortex, suggesting that at least some of the epigenetic changes in the brain of adult subjects with schizophrenia reflect disordered neurodevelopment.

Introduction

Schizophrenia is a complex disorder with a concordance rate of less than 70% in monozygotic twins and non-Mendelian inheritance patterns [1]. While the list of copy number variations, microdeletions and polymorphisms associated with genetic risk for schizophrenia is steadily increasing, straightforward genetic causes are still lacking for a large majority of affected individuals[2–6]. In this context, it comes as no surprise that disease models have been put forward that ascribe an important role for ‘epigenetic’ factors to the pathophysiology of schizophrenia[1,7]. The term ‘epigenetic’ often relates to phenotypes and mechanisms that are the result (or cause) of heritable changes in gene expression and function without any alterations of the DNA sequence [8,9]. More recently, however, ‘epigenetic’ is applied more broadly and at least in the fields of neuroscience and translational medicine, the term often is mentioned in studies focused on chromatin structure and function in dividing and even in postmitotic cells, including neurons[10–12].

Understanding the molecular architecture of chromatin in normal and diseased brain is thought to be important for maladies such as schizophrenia which typically lack a defining cellular pathology but often are associated with alterations in levels of RNAs encoding a

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wide range of transcripts involved in inhibitory or excitatory neurotransmission, myelination and metabolism, among others[12,13]. Because the methylation of DNA at sites of CpG dinucleotides and a wide range of post-translational histone modifications at gene promoters (discussed in detail further below) are involved in the regulation of transcription, these 'epigenetic' markings are often studied in schizophrenia postmortem brain in conjunction with quantification of the corresponding RNA molecules[14–16]. The typical working hypothesis implies that a change in promoter DNA methylation or histone modification is a potential indicator for altered gene expression activity at that locus, which in turn leads to an alteration in the level of the corresponding RNA. From this point of view, chromatin assays and the study of epigenetic markings are a most welcome addendum to the toolbox of the postmortem brain researcher because these methods, when combined with more traditional approaches such as the quantification of mRNA and protein, could provide insight into the mechanisms of gene expression during normal brain development and aging, as well as potential alterations in neuropsychiatric disease, including schizophrenia[12,13].

Several recent excellent reviews summarize our state of knowledge on epigenetic regulation in psychiatric disorders, with the majority of studies published to date primarily focused on preclinical models[10,17–20]. The purpose of this review will be to provide an overview of the chromatin markings that to date were studied in brain of subjects with schizophrenia, including the potential implications for the neurobiology of the disease, and to discuss current limitations and challenges the field needs to overcome.

Chromatin markings reported to be altered in schizophrenia brain

DNA methylation

In vertebrates, the methylation of cytosine residues at CpG dinucleotides within annotated gene sequences is loosely associated with gene expression activity [21,22]. However, within regulatory sequences surrounding transcription start sites and proximal promoters, DNA methylation is often (but not always) thought to mediate, or reflect, transcriptional inhibition[21,22]. To date, most studies exploring DNA methylation changes in schizophrenia postmortem brain were focused on the cerebral cortex, primarily its prefrontal areas. A number of pioneering studies reported various degrees of aberrant CpG hyper- or hypomethylation in regulatory sequences of promoters and genes invoked in the cortical dysfunction of schizophrenia, including the glycoprotein *REELIN*[14,15], catechyl-O-methyltransferase (*COMT*)[23], and *SOX10*, encoding a transcription factor important for myelination and oligodendrocyte function[24]. It is encouraging that these disease-related hyper(hypo)methylation changes were associated with decreased (increased) levels of the corresponding RNA, thereby reiterating the expected function of CpG methylation around transcription start sites. On the other hand, as discussed further below, at least for the *REELIN* and COMT genes, the disease-related DNA methylation changes could not be replicated in independent studies[25,26].

Recently, Petronis' group reported the outcome of the first comprehensive DNA methylation study on the frontal lobe of (for postmortem standards) comparatively large cohorts of 35 schizophrenia, 35 bipolar and 35 control subjects, using an array-based platform that covered CpG enriched sequences positioned at the 5′ end and the promoters of nearly 8000 annotated genes. That study identified altogether approximately 100 loci that –in a surprisingly gender-specific fashion—were affected by a DNA methylation change in the schizophrenia and bipolar cohorts, covering a wide range of gene functions, including glutamatergic and GABAergic neurotransmission and neurodevelopment [25]. The number of loci showing, in comparison to controls, hyper- vs. hypomethylation, was roughly equal, which makes it very unlikely that schizophrenia is associated with a generalized drift towards increased (or decreased) DNA methylation in brain. A similar conclusion was

reached by an earlier study on the temporal cortex of schizophrenia, reporting no significant DNA methylation changes for the entire set of 50 genes examined[27]. Notably, for each of the DNA methylation studies reporting positive findings, the overall magnitude of the disease-related changes, in comparison to controls, were surprisingly subtle. For example, in the Mill et al study, disease-related DNA methylation levels even for one of the most significantly changed genes (*WDR18*) were reported as 17% vs. 25%[25]. While it is difficult, even for single copy genes, to extrapolate the percentages of methylated DNA extracted from a tissue homogenate to the number of cell nuclei affected, the evidence so far would support the view that many of the DNA methylation changes associated with schizophrenia are likely to involve only a small subset of cells residing in the cerebral cortex.

Furthermore, the observation that many of the DNA methylation-related changes in schizophrenia are sex-specific[25] will require additional follow-up studies. Sex steroids may play a role, and there is ample evidence that estrogen-regulated signaling pathways are linked to epigenetic regulation of chromatin, including DNA and histone methylation [1,30,31].

Histone modifications

There are four core histone proteins (H2A, H2B, H3, H4) which comprise an octamer around which 146 bp of genomic DNA are wrapped around; this elementary unit of chromatin fibers is known as the nucleosome. In addition, linker histones, including H1, provide a protein backbone for internucleosomal DNA[32–34]. There is a complex set of posttranslational modifications of specific histone residues located primarily at the N-terminal tail of the core histones. Examples include lysine acetylation and methylation, arginine methylation, serine phosphorylation and also the ubiquitinylation and SUMOlyation of some lysine residues[35,36]. Several of these modifications are thought to be regulated by transcriptional activity, or are involved in epigenetic control of gene expression[37]. For example, lysine acetylation defines chromatin at sites of actual or potential gene expression, while the small molecule modifier, SUMO, is generally associated with transcriptional inhibition[35]. To date, there is only little or no information on the majority of histone modifications in the context of schizophrenia. A study quantifying histone modifications in bulk chromatin from prefrontal cortex reported increased methylation of the histone H3-arginine 17 (H3-methyl-R17) residue in a subset of schizophrenia cases that showed robust deficits in metabolic gene expression[38]. Because the H3-methyl-R17 mark is predominantly expressed in neuronal nuclei[38], one could speculate that some cases with schizophrenia are affected by more generalized changes in certain types of chromatin modifications in neurons and perhaps also in other cells. The latter hypothesis receives further support by other reports describing altered histone acetylation in immunoblot assays from peripheral blood lymphocytes of subjects diagnosed with schizophrenia[39,40]. These more generalized molecular alterations of chromatin in schizophrenia are of interest, and in case of the peripheral blood cells worth to be pursued as potential biomarkers for treatment response[39]. However, it remains to be explored how these more generalized alterations in chromatin modifications relate to the pathophysiology of psychosis.

However, histone modifications can be monitored at specific genomic loci via chromatin immunoprecipitation (ChIP), a technique that fortunately is feasible in postmortem tissue[41–43]. Changes in histone acetylation or methylation in chromatin surrounding disease-related genes were reported for neuropsychiatric conditions, including Friedreich's Ataxia [44]. To date, however, few studies have explored histone modification changes at sites of specific genes in schizophrenia, using either nucleated peripheral blood cells [40] or postmortem brain tissue [16,45,46]as input material. For the latter, preference was given to

the study of lysine methylation which apparently is less affected by tissue autolysis and other postmortem confounds, at least when compared to acetylation[38,41]. The study of lysine methylation is also advantageous given that this type of modification is a key component of the complex regulation of chromatin structure and function (including gene expression), whereby the specific position of the modified lysine residue within the histone protein, and even the numbers of (methyl) groups added to the residue, reflect distinct chromatin states differentiating transcriptionally active ("open") and silenced or repressed loci[12,35,47]. For example, coding and non-coding sequences transcribed by the RNA polymerase II complex are typically defined by sharp peaks of trimethylated H3K4 in nucleosomes positioned around transcription start sites and a more broad distribution of trimethylated H3K36, or dimethylated H3K79, along the transcribed region[48–50]. In contrast, mono-methylated H3K4 defines enhancer sequences potentially further removed from transcription start sites[51]. Furthermore, tri-methylated H3K9, H3K27 and H4K20 are associated with repressive chromatin, while at least in human cells monomethylated H3K9 and H4K20 correlate with gene expression levels[52].

Focusing on the distribution of trimethyl-H3K4 and –H3K27, two chromatin marks differentiating between open and repressive chromatin, respectively, Huang et al. observed that a the prefrontal cortex of a subset of subjects with schizophrenia show, in comparison to matched controls, a shift from the H3K4me3 to the H3K27me3 mark in chromatin surrounding the transcription start site of *GAD1*, encoding 67 KD glutamic acid decarboxylase GABA synthesis enzyme[16]. This observation is of interest given that deficits in GAD67 RNA are considered to be among the most frequently replicated findings in schizophrenia postmortem brain[53], potentially affecting widespread portions of the cerebral cortex, the hippocampus and other areas of forebrain, and even the cerebellar cortex[54–59]. Alterations in GAD67 RNA may also affect cases diagnosed with bipolar disorder and autism[58,60]. Indeed, the shift from H3K4 to H3K27 methylation at the *GAD1* gene in the schizophrenia cases of Huang et al. was accompanied by a deficit in GAD1(GAD67) RNA, in comparison to the matched control[16]. Furthermore, these *GAD1*-associated alterations in prefrontal chromatin of subjects with schizophrenia were influenced by single nucleotide polymorphisms surrounding the 5′ end of *GAD1* previously associated with genetic risk for childhood-onset schizophrenia and other disease, and accelerated loss of cortical gray matter[61–63]. While underlying molecular mechanisms remain to be explored, these findings nonetheless provide some of the first examples how genetic and epigenetic factors could interact and contribute to dysregulated gene expression and eventually, cortical dysfunction and psychosis.

Reproducibility of epigenetic alterations in schizophrenia postmortem brain

Given that, to date, only few studies explored chromatin modifications in schizophrenia postmortem brain, it is difficult to predict whether or not these approaches will contribute to a critical advancement of our knowledge about the mechanisms of disease. As one might expect from an epigenetic marking, recent studies suggest that DNA methylation patterns show, on a genome-wide scale, considerable heterogeneity between individuals, with larger differences between dizygotic as compared to monozygotic twins [64]. Furthermore, the similarities in DNA methylation and histone acetylation patterns among monozygotic twin pairs tend to diminish with age [65]. Given this apparent variability in chromatin markings between individuals, which only increases during the course of aging, it comes as no surprise that to date, the field still awaits independent replication for the reported alterations in schizophrenia postmortem studies. For example, DNA methylation analysis for the *REELIN* promoter (encoding a glycoprotein essential for orderly brain development and neuronal connectivity) reported either no change [25,26] in the cerebral cortex of

schizophrenics, or identified aberrant hypermethylation at different portions of the gene [14,15,66]. Likewise, DNA hypomethylation and increased gene expression for *COMT* (encoding a key enzyme for catecholamine metabolism) was reported in one study[23] while another group reported no methylation changes for the same gene[25]. Besides of interindividual differences, numerous factors could have contributed to these disparate findings. Importantly, the activity of DNA methyltransferases (the enzymes which methylate DNA) and DNA CpG methylation in a variety of tissues, including CNS, are sensitive to the social environment [67,68], ischemia[69], environmental toxins[70–72], nicotine[73,74] alcohol[75], psychostimulants[76,77], and antipsychotic drugs [25,78,79]. It is likely that these factors also play a role in shaping DNA methylation patterns in the human brain, thus further contributing to sample variability in postmortem studies.

Cellular specificity of epigenetic markings

To date, most assays designed to detect and quantify DNA methylation and histone modifications require an input material between 10^3-10^8 nuclei, and this lack of cellular resolution poses a challenge because brain tissue is comprised of an extremely heterogeneous mixture of different cell types. To date, many studies exploring epigenetic dysregulation of gene expression in schizophrenia utilized tissue homogenates for their DNA and histone modification assays, while the gene(s)-of-interest often are expressed only in a select subpopulation of neurons or other cells [14–16,23–27,80]. Some of these changes, such as the hypermethylation of the *REELIN* promoter DNA in cortical layer I of subjects with schizophrenia[15,81,82], or the shift from open chromatin-associated (H3-trimethyl-K4) to repressive histone methylation (H3-trimethylK27) at the *GAD1* gene promoter could indicate an epigenetic defect in the population of inhibitory interneurons[16]. This is a very attractive working hypothesis that could potentially explain the observed deficits in the corresponding REELIN and GAD1(GAD67) RNAs[83]. A more conclusive test of this hypothesis will await the development of technologies that allow the efficient sorting of GABAergic neuron chromatin directly from postmortem tissue. To this end, some progress has been made. For example, it is possible to purify, immunotag (with anti-NeuN antibody) and sort efficiently 10^7 - 10^8 neuronal and non-neuronal nuclei from less than 1 gram of postmortem cerebral cortex in a single day, thereby enabling separate processing of neuronal and non-neuronal chromatin [84,85]. These approaches should be, in principle, also applicable to selected subpopulations of neurons and other cell types. As an alternative, it could be feasible to sort chromatin from tissue homogenates with a set of anti-methyl-histone antibodies that differentiate between open chromatin at sites of actual or potential gene expression, and repressive chromatin, and then assay DNA methylation and other types of modifications separately for these different fractions. This approach was used to show that DNA methylation alterations at the *GAD1* promoter in schizophrenia selectively affect the fraction of repressive chromatin [45].

Implications for the neurobiology of schizophrenia

As discussed above, the study of DNA and histone modifications at defined promoter sequences in schizophrenia postmortem brain is attractive because this approach could potentially uncover epigenetic mechanisms of dysregulated gene expression in that disorder. However, presently it remains an open question whether some of the above mentioned disease-related promoter DNA and histone methylation changes reflect indeed long-lasting and sustained defects in the regulation of gene expression.

Notably, the reported histone methylation changes in prefrontal GAD1 chromatin of schizophrenia subjects [16]are also of interest from the viewpoint of neurodevelopment. There is evidence that H3K4 methylation is progressively upregulated in human prefrontal

cortex at a select set of GABAergic gene promoters (GAD1/GAD2/NPY/SST) during the transitions from the fetal period to childhood and then to adulthood[16]. This extended course of chromatin regulation during the first two decades of life was paralleled by similar increases on the RNA level[16]. Taken together, these findings imply that chromatin remodeling and transcriptional mechanisms involved in the prefrontal dysfunction of schizophrenia also play a role during normal development. Whether the GAD1 chromatin alterations in schizophrenia indeed reflect disordered neurodevelopment, or a type of pathophysiology occurring later in life, remains to be determined in future studies.

From a broader perspective, evidence is emerging that epigenetic markings are highly regulated particularly during late prenatal and early postnatal human brain development. For example, when DNA methylation changes were assayed at 50 gene promoters across the lifespan in human temporal cortex, more than one half of this select set of genes showed age-related methylation changes, which were particularly pronounced during the transition from the fetal period and early childhood years to later stages of development[27]. Likewise, maturation of cerebellar cortex was associated with histone methylation changes in chromatin surrounding glutamate receptor gene chromatin[42]. Future studies that map epigenetic markings on a comprehensive, genome-wide scale during normal human brain development will be necessary in order to find out whether or not these developmental changes are representative for a significant portion of the genome and whether or not schizophrenia-related risk loci and genes show age-related changes. Given the emerging link between childhood adversity, epigenetic dysregulation of gene expression and adult psychopathology[86,87], it is not unlikely that a neurodevelopmental approach will also advance our current understanding of chromatin-associated alterations in brains of subjects with schizophrenia.

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