EPIGENETIC ASPECTS IN SCHIZOPHRENIA ETIOLOGY AND PATHOGENESIS

Nikolay T. Popov, Vili K. Stoyanova, Nadezhda P. Madzhirova, Tihomir I. Vachev

Department of Psychiatry and Medical Psychology, Department of Pediatrics and Medical Genetics, Medical University, Plovdiv, Department of Plant Physiology and Molecular Biology, Plovdiv University, Plovdiv, Bulgaria

ABSTRACT

Epidemiological evidence suggests that etiology of schizophrenia may involve both the influence of genetic factors specific for the individual and the impact of the environment. It is quite likely that a crucial role in the disease development is played by molecular mechanisms mediating the interaction between genes and environment. Modern research have shown that epigenetic mechanisms or chemical modifications of deoxyribonucleic acids (DNA) and histone proteins remain unstable throughout life and can be changed by environmental factors. Thus the epigenetic mechanisms outline an attractive molecular hypothesis of the environment modelling role and the environmental contribution to schizophrenia progression. We give in the present study a general outline of schizophrenia as a pathological entity and discuss the role and involvement of environment versus genetic determinant (nature versus nurture) in the pathophysiological processes. Additionally, we focus on DNA methylation discussing the evidence for the role of that process in schizophrenia. Thirdly, we review the post-translational histone modifications and their role in schizophrenia. These investigations might surely lead further to the development of epigenetic therapy that looks promising in regard to symptom alleviation and the disease-associated cognitive deficit.

Key words: epigenetics, schizophrenia, microRNA molecules, histone modifications

INTRODUCTION

Schizophrenia is a severe disabling disturbance affecting approximately 1% of the population worldwide. The pathologic alterations comprise three main abnormalities: 1) positive symptoms, including hallucinations, delusions and disorganized thinking; 2) negative symptoms, consisting of apathy, anhedonia, abulia and poverty of speech, and 3) cognitive dysfunction, including impaired functioning of memory and overall disorganization. Altogether, these symptoms convert patients into socially isolated and poorly work adaptable persons. Patients with schizophrenia are among the most wronged and maligned members of society. Treatment with antipsychotic drugs only alleviates schizophrenia symptoms and therefore elucidation of etiological mechanisms causing schizophrenic disturbance would probably help the development of novel therapeutical interventions and more effective treatment.

microRNA AND PSYCHIATRIC DISORDERS

Psychiatric disorders are characterized with global dysregulation of multiple signal pathways. Although these conditions may incorporate a genetic component, the variability of patients with seemingly the same type of pathology makes it hard to identify key genes engaged in the development of psychiatric disorders. Probably it also makes the effectiveness of various medications different. These observations suppose engagement of still poorly investigated regulation molecules. Studies on microRNA are focused mainly on schizophrenia and less on bipolar affective disorder. The mechanism of action of the class of small regulatory RNA molecules makes them appropriate for investigation as a potential therapeutical tool in patients suffering from such disturbances.

There is a growing body of evidence that DNA methylation profile can be linked with specific alleles associated with affective disorders. For example
T polymorphism of C677T in methylhydrofolate reductase gene (MTHFR), recently associated with depressive illness.3

**microRNA AND SCHIZOPHRENIA**

Differences in the expression levels of certain microRNA molecules have been found in the brain of schizophrenic patients compared to samples from healthy subjects. Another possible link between microRNA biogenesis and schizophrenia as a neurodegenerative disease has been proposed, investigating the function of DGCR8, which is a key protein in microRNA biogenesis, and is encoded by a segment of chromosome 22, prone to deletion in schizophrenia patients. Thus the impaired biogenesis can be associated with the onset and progress of schizophrenia. Currently, the genes encoding microRNAs, located on the X-chromosome are cloned in order to identify alterations that may be consistent with X-related schizophrenia.4 The results confirm these rare variants in the sequence of mature precursors of microRNA molecules (mRNA-18b, mRNA-502, and mRNA-505) or mature microRNA (let-7f-2, mRNA-188, mRNA-325, mRNA-509-3, mRNA-510 and mRNA-660) are more frequent in male patients, compared to healthy individuals.5 These studies presume that altered microRNA function may contribute to the disease development, but further studies proving the association are needed. The identification of messenger RNA (mRNA) molecules that are targets of these microRNAs is a key step in the formation of an overall idea of the pathways, affected by the proven abnormal expression of protein coding iRNA.

**ROLE OF microRNAs AS BIOMARKERS**

Biomarkers are objectively measurable biologic characteristics which can be used as indicators of normal or pathologic processes. Thanks to recent advances in molecular biology, the range of potential biomarkers has expanded to include genomic profiling, transcriptomic and proteomic analysis. In this regard, microRNA molecules are emerging as a novel class of biomarkers in several diseases, including CNS disorders. In fact microRNA expression profiling has been used to characterize CNS embryonal and differentiated tissues to discriminate cancer from normal tissues, to identify tissue of origin of metastatic cancer. Thus epigenetic modifications and mechanisms constitute a common set of factors called endophenotype.6

**A MODEL OF microRNA ASSOCIATED DYSREGULATION OF THE SYNAPTIC STRUCTURE AND FUNCTION IN SCHIZOPHRENIA**

The activity of the microprocessor complex (ribonucleoprotein complex associated with the processing of microRNA molecules precursors) is increased in the cortical regions as a consequence of the disease-related expression of DGCR8 gene (a gene encoding a protein involved as a subunit in the construction of the microprocessor complex). The acceleration of the processing level results in an increase of the precursors of microRNA molecules, which are exported from the nucleus and processed by Exportin-5 (XPO5 gene encoding exportin-5) and Dicer (RNAase III similar endoribonuclease) enzyme activity. The already exported microRNA molecules are incorporated into RNA-inducing slicing complex (RISC) binding to 3’ non-translationable regions of their target (target transcripts) messengerRNA molecules, encoding synaptic components as neurotrophins/ligands (BDNF, Reelin), neurotransmitter receptors (GRM7, GRIN3A, HTR2A, DRD1) and structural components defining the receptor density of the post-synaptic membrane (DLG4). This interaction reduces the stability of the transcripts and their translational capability. The abnormal levels of microRNA result in a reduction of synaptic protein levels, which in turn leads to alterations in synaptic structure and function.7

**EPIGENETICS AND SCHIZOPHRENIA**

Epigenetic regulators of gene expression, including DNA methylation and posttranslational histone modification might take part in some of the molecular alterations, associated with schizophrenia. For example, in the prefrontal cortex of schizophrenia patients, unusual DNA or histone methylation at site of certain genes or promoters is related to changes in gene expression. These observations are of interest from neurodevelopmental perspective, since increasing amount of evidence for the role of epigenetic modifications of a significant part of the genes and loci, highly regulated in the first years of the development, appear. Additionally, there is indirect evidence that a subset of the antipsychotic drugs, including atypical drugs such as clozapin, interfere with chromatin-remodelling mechanisms.

The challenges in the field include: 1) lack of clear association between the disease and the epigenetic alterations, 2) the lack of cell-specific chromatin analysis, impeding the description of epigenetic alterations in specific cell populations and
3) insufficient knowledge on the stability or turn over of epigenetic “markings” on certain chromatin loci. Despite these disadvantages, studies on DNA changes and histone modifications in chromatin isolated from patients’ and controls’ brain tissue samples, may present precious knowledge on the genetic-epigenetic risk of schizophrenia, especially in the majority of cases with unclear genetic cause.

**HISTONE MODIFICATIONS**

Four main histone proteins (H2A, H2B, H3, H4) constructing the octamer, wrapped by 146 bp. of the genome DNA are known. This unit of the chromatin fibre is called nucleosome. In addition, linking histones such as H1 construct the protein skeleton of the internucleosomal DNA.8-10 A number of post-translational modifications of specific histone residuals are known, located predominantly in the N-terminal tails of the main histones, including lysine methylation and acetylation, arginine methylation, serine phosphorylation, as well as ubiquinitination and binding of SUMO protein to lysine residuals.11,12

It is considered that a part of these modifications can be regulated by the transcriptional activity or are related to the epigenetic control of gene expression.13 For example, lysine residuals acetylation determines chromatin regions with potentially high levels of gene expression, while the modifying molecule, SUMO protein, is usually being associated with inhibition of transcription.11 It is currently insufficient information of only part of the histone modifications, associated with schizophrenia is present. Investigations on the quantitative characterization of histone modification level in prefrontal cortex samples from schizophrenia patients and controls show increased methylation of histone H3-arginine 17 (H3-methyl-R17) residuals in majority of the cases, showing unambiguous deficit in the process of gene expression.14 Since H3-methyl-R17 modification is found predominantly in the neuron nucleus14, it might be assumed that some of the schizophrenia cases are characterized with more generalized alterations in some of the types of chromatin modifications in neurons and other cells. The latter hypothesis is additionally supported by other investigations, describing altered histone acetylation in experiments with immunoblot of peripheral blood lymphocytes from schizophrenic patients.15,16 While these basic molecular chromatin alterations in schizophrenia remain of interest, investigating peripheral blood cells is convenient in the light of their use as a substrate in the search of potential diagnostic biomarkers.15

**STUDIES ON SCHIZOPHRENIA INHERITANCE**

The evidence for disease aggregation in certain families shows the presence of inheritance component. In fact, there are serious studies supporting the family component in schizophrenia. The risk of schizophrenia and related disorders in first-degree relatives is approximately 9 times greater than the risk of schizophrenia in the general population.17,18 The degree of genetic identity to the proband is strongly related to the degree of risk, and consequently the first-degree relatives are at higher risk of psychosis compared to more distant relatives of the affected individuals.14

Twin studies are ordinarily used in the assessment of a disease inheritance. Extensive investigations on twins with schizophrenia have shown that the schizophrenia risk in a sibling (co-twin) of the proband is significantly higher in identical (monozygotic, 53%) than in dizygotic (15%) twins, so that the genetic predisposition to schizophrenia has been assessed at 68%.19,20 The monozygotic discordance may be explained by the reduced penetrance of the schizophrenic genotype, which is supported by the observation that a higher risk of schizophrenia is present in the offspring of the unaffected twins of discordant monozygotic pairs (nephews).19,20 A possible explanation of these observations may be attributed to environmental factors, altering gene function, which on its turn creates vulnerability to schizophrenia.21

**CONCLUSION AND FUTURE DIRECTIONS**

Currently, it is becoming clear that the epigenetic modifications not only present molecular mechanisms, responsible for significant changes in the behaviour and the brain function, but also play a significant role in the dynamic character of the adult CNS in response to the environment. In that manner, the epigenetic basis of schizophrenia turned into an attractive molecular hypothesis, supported by numbers of evidence up to this moment. Unfortunately, post-mortem studies are not able to show if the epigenetic mechanisms are causative to schizophrenia. Therefore, model species like rodents would be probably introduced in the future studies of neuropsychiatric disorders. One of the most important questions to be addressed by future studies is the capability of epigenetic treatment of alleviating the cognitive deficit caused by schizophrenia. The fact that histone methylation may be important for the early diagnosis of some schizophrenia cases, shown by post-mortem studies on gene expression...
alterations, is encouraging. Additionaly, the pharmacogenetic models for schizophrenia treatment are of great importance. Up to the present, limited clinical and scientific data on the role of DNMT (DNA methyltransferase) and HDAC (histone deacetylase) inhibitors in the alleviation of some molecular and behavioural manifestations of rodent cognitive dysfunction, is present. Nevertheless, beneficial effects of the inhibition of DNMT or HDAC have been observed in rodents in terms of relaxation of some molecular and behavioural manifestations of cognition dysfunction. Such observations are promising for the potential of the drugs, able to change chromatin characteristics, as an applicable therapy in schizophrenia treatment. The studies on epigenetic modifications in the process of normal brain development, regulation of normal cognition, and in post-mortem tissue samples, bring hope to the future, when the molecular mystery underlying the disease will be solved. Elucidating of the pathophysiological mechanisms would certainly facilitate the development of more effective therapeutic strategies.

REFERENCES
ЭПИГЕНЕТИЧЕСКИЕ АСПЕКТЫ ЭТИОЛОГИИ И ПАТОГЕНЕЗА ШИЗОФРЕНИИ

Н. Попов, В. Стоянова, Н. Маджирова, Т. Вылчев

РЕЗЮМЕ

Эпидемиологические исследования демонстрируют одновременно влияние специфического генетического фактора индивида и воздействие окружающей среды на этиологию шизофрении. Молекулярные механизмы, определяющие взаимодействие между генами и окружающей средой, по всей вероятности играют значительную роль в процессах развития заболевания. Современные исследования показывают, что эпигенетические механизмы или химические модификации дезоксирибонуклеиновых кислот (ДНК) и гистоновых протеинов остаются лабильными во время жизни и могут изменяться под влиянием факторов окружающей среды. Таким образом эпигенетические механизмы очерчивают привлекательную молекулярную гипотезу относительно моделирующего участия окружающей среды и ее вклада в развитие шизофрении.

В настоящей работе: 1. Представляется общий обзор шизофрении как заболевания; обсуждаются роль и участие среды в отношении генетической детерминанты (nature versus nurture) в процессе патологии. 2. Авторы сосредоточивают свое внимание на метилировании ДНК (обсуждаются доказательства о роли этого процесса при шизофрении). 3. Рассматриваются посттрансляционные гистоновые модификации и их роль при шизофрении.

В будущем эти исследования могут привести к развитию эпигенетической терапии с обещающими результатами для облегчения симптоматики и когнитивного дефицита, ассоциированного с заболеванием.