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Appraisal of state-of-the-art

Preclinical epigenetic models for screening epigenetic drugs for schizophrenia

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ABSTRACT

Schizophrenia is an important psychiatric disorder for which effective drugs are available. However, there are problems with current drug therapy of schizophrenia in that some patients do not respond adequately. Moreover, some patients show treatment resistance and some patients show cognitive decline despite treatment. Hence new and effective drugs will be useful for the treatment of this disorder. Since there is increasing evidence that epigenetic mechanisms of gene expression are defective in schizophrenia, drugs that correct epigenetic defects, epigenetic drugs, could be useful in the treatment of this disorder. This paper discusses preclinical epigenetic models for screening epigenetic drugs for schizophrenia. It also discusses how such models could be useful for the discovery and development of such drugs.

1. Introduction

Schizophrenia is a common disorder which exerts a great toll medically and financially on affected patients, their families, and society. Despite more than a century of active research, the pathogenesis of schizophrenia remains obscure. Family, twin, and adoption studies have established that hereditary factors are involved in the development of schizophrenia. Despite decades of intensive search by genetic mapping studies, till date no genetic mutation or polymorphism has been definitively identified that predisposes to this disorder (Fosse, Joseph, & Richardson, 2015; Grayson & Guidotti, 2013), although some potentially important genes that may be associated with the pathogenesis of schizophrenia have been identified (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Another possibility for the inheritance pattern of schizophrenia involves epigenetics, a term which means above or in addition to genetics. Epigenetics is presently an active area of research all over the world and involves molecular mechanisms like DNA methylation, histone modifications, and RNA-mediated regulation of gene expression. There is increasing evidence that epigenetic mechanisms of gene expression are involved in the pathogenesis of schizophrenia (Shorter & Miller, 2015). A partial list of genes found to be epigenetically modified in schizophrenia patients is given in Table 1. In the light of the possible role of epigenetic mechanisms in the pathogenesis of schizophrenia, this article discusses the value of preclinical epigenetic models for screening epigenetic drugs for treating schizophrenia, comparing them with the older, more traditional preclinical models of this disorder.

2. Preclinical epigenetic models for screening drugs for schizophrenia

The preclinical epigenetic models for screening drugs for schizophrenia are listed in Table 2. They have been recently discussed in detail by Peedicayil, Dong, and Grayson (2014). Hence they will only be briefly discussed here. They comprise in vivo and in vitro models.

2.1. In vivo preclinical epigenetic models of schizophrenia

2.1.1. L-methionine-induced hypermethylation model

One in vivo model is the L-methionine-induced hypermethylation (MIH) model. This model was first described by Tremolizzo et al. (2002) who found that when L-methionine was administered to mice for 15 consecutive days, the mice showed significant downregulation of the expression of *RELN* and *GAD1* mRNAs compared with control mice. The levels of 400- and 180-kDa reelin immunoreactive proteolytic fragments were also significantly decreased in the L-methionine-treated mice. These findings are relevant to schizophrenia since the *RELN* and *GAD1* genes have been shown to be down-regulated due to hypermethylation in the post-mortem brain of schizophrenia patients (Table 1). Later, the same group (Tremolizzo et al., 2005) studied the behavioral effects of prolonged administration of L-methionine to mice. The authors found that mice which were treated with L- methionine, when compared with control mice, showed decreased social interaction in the home cage, decreased social interaction in a new environment, reduced social isolation-induced social aggression, and impaired prepulse inhibition of the startle reflex. The L-methionine-induced behavioral features of mice have similarities with the clinical features of patients with schizophrenia. The authors also found that valproate,

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Table 1
Partial list of genes found to be epigenetically modified in schizophrenia.

Gene	Gene product	Epigenetic change	Tissue	Reference
RELN	Reelin	DNA hypermethylation	P-M brain	Grayson and Guidotti (2013) Guidotti and Grayson (2014)
GAD1	Glutamic acid Decarboxylase	DNA hypermethylation	P-M brain	Grayson and Guidotti (2013) Guidotti and Grayson (2014)
BDNF	Brain-derived Neurotrophic factor	DNA hypermethylation	P-M brain	Guidotti and Grayson (2014)
mGlu2	Metabotropic Glutamate receptor 2	DNA hypermethylation	Peripheral blood	Kordi-Tamandani, Dahmardeh, and Torkamanzehi (2013)

Abbreviation: P-M: post-mortem.

which is known to act as a histone deacetylase inhibitor (HDACi), enhanced acetylated histone3 content, and prevented L-methionine-induced *RELN* promoter hypermethylation, reelin mRNA downregulation and impaired prepulse inhibition of the startle reflex. In this context, HDACi like valproate are also thought to induce demethylation by activating DNA demethylating mechanisms (Guidotti & Grayson, 2014).

2.1.2. Prenatal restraint stress model

Another in vivo epigenetic model of schizophrenia is the prenatal restraint stress (PRS) model, which can involve mice and rats. Matrisciano, Tueting, Maccari, Nicoletti, and Guidoti (2012) developed a mouse model in offspring of mothers restrained during pregnancy. The pregnant mothers were restrained in a 12 × 3 cm transparent tube under bright light for half an hour twice a day from day 7 of pregnancy until delivery. Control mothers were not disturbed throughout pregnancy. After weaning (postnatal day 21) male mice were studied further, and housed separately. Behavioral tests were conducted to study social interaction, locomotor activity, and prepulse inhibition of startle reflex. After the mice were sacrificed, mouse frontal cortex tissue was studied for biochemical and molecular biological parameters. The authors found that mice that underwent PRS exhibited decreased expression of metabotropic glutamate 2 and 3 (mGlu2 and mGlu3) receptors in the frontal cortex from day 1 of postnatal life. These data are of interest because the gene encoding the mGlu2 receptor has been found to be hypermethylated (which leads to decreased expression of the receptor) in DNA obtained from peripheral blood of schizophrenia patients (Table 1). The mice also showed altered brain-derived neurotrophic factor (BDNF), glutamic acid decarboxylase (GAD1), and DNA methyltransferase 1(DNMT1) mRNA expression. These data are relevant to schizophrenia since the *BDNF* gene has been shown to be down-regulated due to DNA hypermethylation in the post-mortem brain in schizophrenia patients (Table 1). The mice also exhibited alterations in social interaction, locomotor activity, and prepulse inhibition of the startle reflex. Matrisciano et al. (2012) suggested that these findings represent behavioral and biochemical changes similar to those seen in schizophrenia. Maccari et al. (1995) described a PRS model in rats. They divided pregnant Wistar rats into two groups. One group acted as control and was left undisturbed in the home cage. In the other group, pregnant females were placed individually in a plastic transparent cylinder 6 cm in diameter and 20 cm long for three 45 minute periods every day between days 14 and 21 of pregnancy. After delivery, half the pups were raised by the biological mother, and half were allotted to either control or prenatally stressed foster mothers. The pups were kept in the adoptive mother's cage within 3 to 6 h after birth. During this procedure, the mothers were removed from their cages briefly. The pups were weaned 21 days following birth and left without disturbance till testing at 90 days of age. The authors found that in adult rats PRS prolonged the secretion of corticosterone induced by restraint stress. It was also found that PRS reduced hippocampal corticosteroid receptors. Adoption at the time of birth was found to totally reverse PRS effects on corticosterone secretion and hippocampal receptors. Adoption was also found to increase maternal behaviour since foster mothers spent more time licking and picking up pups compared to biological mothers. The time taken to replace all pups in the cage was also less among the foster mothers compared to the biological mothers. Hence, this study showed that prenatal and postnatal events have long-term effects on the hypothalamo–pituitary–adrenal (HPA) axis. These data are of interest because epigenetic changes associated with the HPA axis may be a link between stress in early life and the development of schizophrenia later in life (Ruby et al., 2014).

Table 2
Preclinical epigenetic models for screening drugs for schizophrenia.

In vivo epigenetic models:
L-methionine-induced hypermethylation (MIH) model
Prenatal restraint stress (PRS) model: mice and rats
In vitro epigenetic models:
Neuronal precursor cells (NT2 cells)
Primary cortical and hippocampal neuronal cultures
Induced human pluripotent stem cells

Adapted with permission from Peedicayil et al. (2014).

2.2. In vitro preclinical epigenetic models of schizophrenia

2.2.1. Neuronal precursor cells

In vitro epigenetic models include those based on cell culture. One such model is the NT2 neuronal precursor cell model. NT2 cells are a cell line obtained from a human male germ cell carcinoma, and are one of the best stem cell lines that can be made to differentiate into neurons and astrocytes. The cell line can be made to differentiate into neuronal cells by incubating it with retinoic acid. Chen, Sharma, Costa, Costa, and Grayson (2002) kept NT2 cells in Dulbecco's Modified Eagle Medium/F-12 nutrient mixture, 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and glutamine. Differentiated neurons were induced from the NT2 cells by treatment with retinoic acid for up to 6 weeks. The authors treated low-density cultures of NT2 cells with decitabine, a DNA methyltransferase inhibitor (DNMTi) at many concentrations, and trichostatin A, a histone deacetylase inhibitor (HDACi) for various time periods. The cells were then harvested for total RNA isolation. NT2 cells were also treated with different concentrations of valproic acid and the inactive amide of valproic acid, valpromide, for 40 h and then harvesting was performed for analysis of RNA. It was found that the *RELN* promoter was more methylated when this gene was silent. Moreover, activation of this gene by the addition of retinoic acid, decitabine, valproic acid and trichostatin A was associated with a reduction in the methylation of the promoter of *RELN*. Kundakovic, Chen, Costa, and Grayson (2007) later studied the effects of the DNMTi doxorubicin, azacytidine, and zebularine on the expression of the *RELN* and *GAD1* genes in NT2 cells. They found that