Translational Research in Neurodevelopmental Disorders: Development of Etiology-Based Animal Models

Genetic Animal Models of Schizophrenia Related with the Hypothesis of Abnormal Neurodevelopment

Lingling Lu, Takayoshi Mamiya, Takenao Koseki, Akihiro Mouri, and Toshitaka Nabeshima

The Academic Frontier Project for Private University, Comparative Cognitive Science Institute, Meijo University; Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University; and Japanese Drug Organization for Appropriate Use and Research; Nagoya 468–8503, Japan.

Received January 4, 2011

Accumulating evidence supports the existence of an overlap in genetic susceptibility with schizophrenia. Translation of human genetic mutations into animals is one of the most important strategies to study the pathogenesis of schizophrenia, identify potential drug targets, and test new medicines for antipsychotic treatment. Recent discoveries of susceptibility genes for schizophrenia make the possibility to develop newer genetic mouse models based on the neurodevelopmental hypotheses of schizophrenia. Although it is not possible to mimic all schizophrenic symptoms by these animal models, the genetic mouse models based on the neurodevelopmental hypothesis are widely developed to reproduce several schizophrenia-like behavioral and biochemical changes in humans. In this mini review, we will discuss the neuropathological and behavioral manifestations of representative genetic mouse models for schizophrenia, associated with the hypothesis of abnormal neurodevelopment.

Key words schizophrenia; genetic mouse model; neurodevelopmental hypothesis

1. INTRODUCTION

Schizophrenia is a heritable mental disorder characterized by chronic psychotic symptoms and cognitive deficits. For more than three decades, the neurodevelopmental hypothesis has prevailed for schizophrenia. This hypothesis posits that schizophrenia is the behavioral outcome of an aberration in neurodevelopmental processes that begins long before the onset of clinical symptoms and involves deficits in the genetic program for normal formation of synapses and migration of neurons, as well as their connections in brain development.

With growing evidences on the neurobiology and genetics of schizophrenia, more animal models have been developed to study the molecular mechanisms of pathophysiological changes and to design more effective therapies for schizophrenia. In principle, genetic manipulation offers advantages over pharmacologic models because it is more selective in its effects and provides a means to alter their expression during transduction pathways that take critical roles in brain development.

In this article, we briefly review the genetic animal models related with the hypothesis of abnormal neurodevelopment of schizophrenia at present.

2. GENETIC EVIDENCE OF NEURODEVELOPMENTAL HYPOTHESIS

Schizophrenia is a clinically heterogeneous psychotic illness whose etiology remains poorly understood. However, clinical, epidemiological, genetic, and neuropathological features of schizophrenia continue to suggest that abnormal neurodevelopment is important for the disorder. Genetic studies have identified several specific genes that are associated with schizophrenia risk in a number of populations. Generally, twin studies have shown that schizophrenia is a predominant genetic disorder, with estimates of heritability risk ranging at 50—60% and there are recent reports of 80%. Family studies have found that single effects of a major gene are unlikely; instead, polygenic models effects of multiple-risk genes acting additively or multiplicatively may provide the best explanation for schizophrenia. Several neurodevelopment-related genes are located in chromosomal loci that are associated with potential candidate genes due to their polymorphic status, and to alter their expression during embryonic stages. They might putatively result in the best explanation for schizophrenia. Hence identification of genes responsible for this high heritability will be critical to understanding this disorder. Moreover, without parsing based on genotypes, the environmental and epigenetic factors may be more difficult to clarify.

Anatomical abnormalities such as ventricular enlargement, volume reductions of prefrontal cortex and hippocampus, and generalized brain reduction are well established among other features of schizophrenia. Obvious alterations in neuron size and morphology as well as synaptic connectivity are also observed in schizophrenic patients. Investigations focusing on cortical and limbic brain regions increasingly demonstrate that structural and molecular integrity of the synaptic complex, glutamate-related receptors, and signal transduction pathways take critical roles in brain development.
ment, synaptogenesis, and synaptic plasticity. More importantly, most schizophrenia-related specific genes including disrupted in schizophrenia-1 (DISC1), neuregulin-1 (NRG1), dysbindin-1, and AKT-1 play important roles in neurodevelopment, neurotransmission, and neuroplasticity. Although variations among each gene confer only modest increases in the risk for schizophrenia, the discovery of these genes and identification of candidate proteins and molecular pathways may importantly contribute to the pathophysiology of schizophrenia.

3. GENETIC ANIMAL MODELS RELATED TO THE NEURODEVELOPMENTAL HYPOTHESES OF SCHIZOPHRENIA

Schizophrenia-Susceptibility Genes. Disrupted in Schizophrenia-1 (DISC1) Schizophrenia susceptibility genes have been identified by human genetic studies and enabled us to generate mouse models on the basis of genetic etiology. Because causal mutations have not been identified, there is still debate on the significant roles of each gene. Nonetheless, many of the genetically engineered models for these genes display behavioral abnormalities and morphological alterations that may be relevant to schizophrenia patients. Here, we summarize the behavioral and morphological changes in representative genetic mouse models that are associated with the neurodevelopmental hypotheses of schizophrenia.

Numerous studies have found that DISC1 is highly expressed during brain development and plays critical roles in the growth of the embryonic and postnatal brain. It has been confirmed that DISC1 affects the development and maturation of neuronal systems, which is implicated with psychiatric disorders. DISC1 was originally identified as truncated by a translocation that segregated with schizophrenia. Therefore several transgenic animal models expressing this truncated protein have been generated. In several kinds of transgenic mice expressing truncated DISC1, enlarged lateral ventricles, reduced cortical thickness, and partial agenesis in the corpus callosum as well as reduced immunoreactivity of parvalbumin (PV) in both the medial prefrontal cortex (mPFC) and the hippocampus have been detected as important hallmarks for schizophrenia. Furthermore, transient expression of a dominant-negative DISC1 (C-terminal fragment of DISC1) at postnatal day 7 (PD 7), reduces the hippocampal synaptic transmission as a result of decrease of the hippocampal dendritic complexity. In another type of DISC1 transgenic mice, misoriented and shorter dendrites and decrease in numbers of synaptic spines of hippocampal granule cells have been found, which cause reduced short-term potentiation at CA3/CA1 synapses and indirect working memory deficit.

Recent study has revealed critical roles of DISC1 in regulation of the embryonic and adult neurogenesis. DISC1 is highly expressed in the embryonic ventricular/subventricular zones of the cortex where neural progenitor cells reside, suggesting that this gene regulates their proliferation and/or differentiation. To confirm this idea, DISC1 RNA interference (RNAi) was introduced into neural progenitors in the developing neocortex, using in utero electroporation, and a significant reduction of proliferation of progenitor cells and differentiation of premature neurons was observed. Interestingly, overexpression of DISC1 in neural progenitors results in an opposite phenotype. It has been also found that knockdown of DISC1 using a lentivirus to deliver RNAi results in a decrease in the proliferation of adult progenitor cells in the dentate gyrus. Moreover, such transgenic DISC1 mutant mice show hyperlocomotion in a novel environment and increased immobility time in a forced swimming test, which are frequently observed in animal models displaying schizophrenia-like behavior.

In our recent study, by utilizing the methods of in utero electroporation, we have successfully generated a novel mouse model by transferring RNAi of DISC1 at embryonic day 14 (E 14) selectively to disrupt DISC1 gene expression in a lineage for pyramidal neurons mainly in the prefrontal cortex during neurodevelopment. Our results indicate that knockdown of DISC1 leads to maturation-dependent deficits in mesocortical dopaminergic projections and induces a series of schizophrenia-like behavioral abnormalities including hyperlocomotion in a novel environment, enhanced immobility time in forced swimming test, deficits in prepulse inhibition (PPI) of startle response, and memory impairments in novel object recognition test after sexual maturation. Importantly, these behavioral abnormalities are attenuated by treatment with atypical antipsychotic clozapine, indicating their association with schizophrenia. Moreover, we further observed a significant decrease in the extracellular level of dopamine and tyrosine hydroxylase (TH), a marker of mature axonal terminals of the dopaminergic projection, and disturbances of PV interneurons and pyramidal neurons in mPFC of DISC1 knockdown mice at PD 56 but not PD 28 or PD 42. These results suggest that DISC1 may play critical roles in neurodevelopment and its disruption during development may induce several schizophrenia-like features in adult mice.

Although disruption of DISC1 may be critically involved in many cases of schizophrenia, direct evidence from genetic linkage and association studies suggests that variants of DISC1 may not occur in most cases of the disorder, compared with other psychotic diseases.

Neuregulin-1 (NRG-1) NRG-1 was first identified as a susceptibility gene for schizophrenia in an Icelandic population and further confirmed by subsequent studies. NRG-1 plays important roles in brain development, such as neuronal migration and neurite outgrowth, as well as proliferation of glia cells. Furthermore, increased levels of NRG-1 type I mRNA are observed in schizophrenia patients. Tissue culture study has revealed that NRG-1 dampens N-methyl-D-aspartate (NMDA) receptor function in pyramidal neurons of the prefrontal cortex. Therefore NRG-1 mutant is considered a useful in genetic animal model for schizophrenia.

NRG-1 transgenic mouse models have been generated by manipulation of expression of different NRG1 isoforms by several studies. Several schizophrenia-like behaviors, such as hyperlocomotor activity and impaired PPI, as well as decreased expression of NMDA receptor have been observed in heterozygous NRG-1 knockout mice lacking the transmembrane domain of NRG-1 gene. Moreover, disruption of type III NRG1 in adult mice results in increased volume of lateral ventricles and decreased density of dendritic spines in...
hippocampal pyramidal neurons.36) Knockout of the NRG-1 receptors ErbB2 and ErbB4 (ErbB2/4) at early embryonic stages decreases the density of spine in both the cortex and hippocampus.37) In behavioral analysis, ErbB2/4 knockout mice display an increase in aggression and a deficit in PPI, as a model of sensorimotor gating that is abnormal in schizophrenia patients.37) Moreover, ErbB4 knockout mice show a decrease in the power of kainate-induced gamma oscillations38) and reduction of the density of calbindin-positive GABAergic interneurons in the cortex, as well as PV-positive interneurons in the hippocampus.39)

Most positive single nucleotide polymorphisms (SNPs) are located upstream of the start site of NRG-1 exons, which suggests a probable effect on its expression. Whereas, the consequences of mutations in the region of NRG-1 remain unknown, and whether the heterozygotes knockout resembles a disease state is arguable. In addition, NRG-1 comprises in at least six major isoforms and many splice variants. The alterations of which one is particularly important for schizophrenia remain unclear, but they are quite critical to design genetically engineered animal models related to this disorder.

**Dysbindin** Dysbindin is another likely susceptibility gene that has been identified by several studies.40,41) Irish study of high-density schizophrenia families has suggested that schizophrenic patients with negative symptoms are more likely to inherit the risk of dysbindin mutant, raising the possibility that negative symptoms in psychotic bipolar cases of schizophrenia are likely attributable to heritability of dysbindin mutation. Postmortem study has reported a decrease in the level of gene transcription of dysbindin and its protein expression in brain tissues of schizophrenia patients.42)

Thus sandy (Sdy) mouse has been designed to mimic a deficiency of dysbindin in human.43) As reported, Sdy mouse harbors a spontaneously occurring deletion in the DTNBP1 gene and expresses no dysbindin protein, which provides a unique tool to study the role of dysbindin in schizophrenia. Sdy mice also exhibit morphological changes in excitatory asymmetrical synapses on hippocampal CA1 dendritic spines, larger vesicle size, slower vesicle release, and lower release probability, as well as smaller total population of the readily releasable vesicle pool.44) These mutant mice display deficits of neurosecretion and synaptic morphology in hippocampal neurons, and manifest some schizophrenia-like behavior such as social withdrawal and cognitive deficits. In the hippocampus of Sdy mice, the level of Snapin (a SNAP25-binding protein and a synaptic priming regulator) is reduced, which suggests that destabilization of Snapin in the Sdy mice may lead to abnormal neurotransmission and abnormal behavior.45) Although more information for the associations between dysbindin gene and schizophrenia is needed, Sdy mice are able to serve as a genetic animal model to identify potential pathways of dysbindin in schizophrenia.

**Brain-Derived Neurotrophic Factor (BDNF)** BDNF has been found to play important roles in promoting and modifying growth, development, survival of neuronal populations, and activity-dependent neuronal plasticity.46) BDNF is implicated in the pathogenesis of schizophrenia, since its expression is reduced in some postmortem brains of schizophrenia patients,47,48) indicating that the downregulated expression of BDNF leads to abnormalities in developing brain. Atypical BDNF knockout mice, in which one allele of BDNF gene is disrupted through the whole developmental stages, exhibit hyperactivity in locomotion and behavioral deficits in spatial learning and memory.49,50) Dysfunction of non-spatial associative memory is also observed in conditional BDNF knockout mice, in which BDNF gene is disturbed approximately 3 weeks.51) However, these mutant mice do not show hypersensitivity or deficits in PPI and fear conditioning.52,53) Moreover, dysfunction of context-dependent fear memory has been found in inducible BDNF knockout mice, in which the disrupted BDNF gene is limited in specific brain regions at certain developmental stages.54) Further inducible knockout of BDNF from the embryonic stage induces severer context-dependent memory deficits compared with later knockout mice. These data suggest that BDNF plays a critical role in neurodevelopment. However, the genetic linkage between BDNF and schizophrenia is relatively weak, although the biochemical and behavioral changes in BDNF knockout mice resemble several pathological changes of schizophrenia.55,56)

**Reelin** Reelin is a glycoprotein that guides neurons and radial glial cells to corrected position in the developing brain. A series of studies suggest that reelin might be a vulnerability gene involved in the development of psychosis including schizophrenia.57—59) “Reeler mice” is a naturally occurring mutant mouse model generated by disrupting the reelin gene. These mice exhibit decreased expression of reelin and glutamic acid decarboxylase 67 (GAD67), as well as lower density of dendritic spine,57) which resemble some pathological changes of schizophrenic patients.58) Moreover, these mutant mice show behavioral deficits in PPI, which is specifically involved in schizophrenia, although the reelin mutant mice do not show abnormalities in working memory or social interaction. Nonetheless, the reelin gene appears to play important roles in neuronal development and could mediate outcomes of some causative mutations in other genes and critical environmental insults.

**NMDA Receptor Subunit 1 (NR1)** Genetic disruption of NR1 by traditional and conditional knockout in mice results in hyperlocomotion, stereotypy, abnormal social behavior, cognitive dysfunction, and abnormal brain development.60—62) These abnormalities resemble several aspects of schizophrenia. Importantly, some deficits are attenuated by antipsychotic treatment. Roles of NR1 in the pathology of schizophrenia are further supported by decreased expression of NR1 in postmortem tissues from schizophrenic patients and an increase in NR1 expression by chronic antipsychotic treatment.63—65) Small molecules that enhance the function of NMDA receptor are being tested as novel adjunct therapies for schizophrenia treatment in clinical trials.66,67)

Recently, Belforte et al.68) characterized a mouse strain in which the essential NR1 subunit of the NMDA receptor is selectively eliminated by 40—50% in cortical and hippocampal interneurons in early postnatal development. Consistent with the NMDA receptor hypofunction hypothesis of schizophrenia, the postnatal NR1-ablated mice exhibit distinct schizophrenia-related symptoms after adolescence, including novelty-induced hyperlocomotion, mating and nest-building deficits, as well as anhedonia-like and anxiety-like behaviors. In addition, impairment of social memory, spatial working memory, and prepulse inhibition are also observed in the mutant mice. Furthermore, reduced expression of GAD67 and
PV is accompanied by disinhibition of cortical excitatory neurons and reduced neuronal synchrony. However, postadolescent deletion of NR1 did not result in such abnormalities, suggesting that early postnatal inhibition of NMDA receptor activity in corticodendritic GABAergic interneurons contributes to the pathophysiology of schizophrenia-related disorders.68)

Others Studies have focused on a number of interesting candidate genes in neurodevelopmental hypotheses of schizophrenia, such as neural cell adhesion molecule (NCAM), cyclin-dependent kinase-5 (CDK5), V-akt murine thymoma viral oncogene homolog 1 (Akt1), Lis1, and Lhx5 so as to prepare potential genetic models.69—72) Mice expressing these mutant genes show abnormal development in the brain and some schizophrenia-like behavior, although the patterns of abnormality vary. Clearly, the potential genetically to manipulate genes that affect brain development and to explore phenomenological links to the molecular and behavioral phenotypes related to schizophrenia is virtually limitless.

4. PERSPECTIVES

Schizophrenia has been long recognized as a heritable mental illness that probably involves multiple genes with relatively modest effects across large populations.73) Most of these identified susceptibility genes such as DISC-1, NRG-1, AKT1, and Reelin are known to have essential functions in neurodevelopment including neuronal differentiation, migration, survival, synaptogenesis, and apoptosis.27,74—77) Thus some aspects of altered brain development in schizophrenia may be attributable to abnormal expression of genes that are essential for early neurodevelopmental processes. Furthermore, such genetic mechanisms may significantly interact with prenatal and/or perinatal environmental insults to enhance the risk of developing schizophrenia.

Until now, there is still debate whether it is possible to use rodent models to reflect psychiatric disorders in humans. However, genetically engineered mice in which susceptibility genes are modified have potential advantages over human studies. In the case of schizophrenia, initial risks for this disorder occur during neurodevelopment, whereas onset of the disease arises in adulthood, with almost two decades for the full development of pathology to overt incidence. To understand in detail the mechanisms of schizophrenia, it is important to characterize how the disorder etiologies develop over time until development of full-blown disease. Therefore genetically engineered mouse models may be expected to provide further understanding of the disease mechanisms and time course. Another major advantage of genetic mouse models is their usefulness for compound screening in drug development, since rodents are much easier for preclinical drug screening from both economical and ethical viewpoints. For these reasons, genetic mouse models may provide an opportunity to identify novel therapeutic strategies that are directly linked to the mechanisms of psychological disorders.

Many investigators have considered the possibility that brain function and behavior are modulated by a combination of several genetic and environmental factors, and the concept of “pathway” is more likely to mimic the mechanisms than the effect of a single gene product. Therefore it is necessary to co-transfer more than one gene, such as by the methods of in utero electroporation.28,78,79) This will make it possible to evaluate the synergistic influence or epistatic effect of multiple genetic factors, as well as to test how defects in neuronal network formation in early development lead to the behavioral abnormalities in adulthood.

In summary, we tried to establish a series of abnormalities in genetically engineered mice models based on the neurodevelopmental hypotheses of schizophrenia. It seems clear that the multiple similarities of genetic mouse model with schizophrenic patients indicate the potential for further understanding the pathogenesis of schizophrenia. Generation of these genetic mouse models should shed light on the etiology of schizophrenia and lead to more effective therapies in the future.

Acknowledgements This study was supported by Grants-in-Aid for Scientific Research (A) (22248033), Scientific Research (B) (20390073) (21390045), and Exploratory Research from the JSPS (19659017) (22659213) by the “Academic Frontier” Project for Private Universities (2007—2011) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT); by Regional Joint Research Program supported by Grants to Private Universities to Cover Current Expenses from MEXT, by Research on Regulatory Science of Pharmaceuticals and Medical Devices from the Ministry of Health and Labour and Welfare (MHLW); by Research on Risk of Chemical Substances, Health and Labour Science Research Grants supported by MHLW and by joint research project under the Japan–Korea basic scientific cooperation program by Japan Society for the Promotion of Science (JSPS). We thank Dr. Ping Lu for revising the manuscript.

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