Received: 18 December 2017 | Revised: 12 February 2018 | Accepted: 13 February 2018

DOI: 10.1111/cns.12840

REVIEW ARTICLE

WILEY CNS Neuroscience & Therapeutics

Dysregulation of miRNA and its potential therapeutic application in schizophrenia

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 81372688, 81373382 and 81773702

Summary

Although it is generally believed that genetic and developmental factors play critical roles in pathogenesis of schizophrenia, however, the precise etiological mechanism of schizophrenia remains largely unknown. Over past decades, miRNAs have emerged as an essential post-transcriptional regulator in gene expression regulation. The importance of miRNA in brain development and neuroplasticity has been well-established. Abnormal expression and dysfunction of miRNAs are known to involve in the pathophysiology of many neuropsychiatric diseases including schizophrenia. In this review, we summarized the recent findings in the schizophrenia-associated dysregulation of miRNA and functional roles in the development and pathogenesis of schizophrenia. We also discussed the potential therapeutic implications of miRNA regulation in the illness.

KEYWORDS
miRNAs, neuroplasticity, schizophrenia, single nucleotide polymorphism

1 | INTRODUCTION

Schizophrenia is a disabling disorder characterized by complex symptoms of abnormal social behavior, mental disturbance, thinking disruption, and impaired cognition. It affects nearly 1% of the world population.[1] It is generally believed that the interplay between developmental and environmental factors contributes to the pathogenesis of this disease; however, the precise etiology remains unknown. From the view of pathophysiology, abnormal neurotransmission, particularly disturbed dopamine-glutamate transmission and altered prefrontal dysfunction may play an important role in the majority of the symptoms of schizophrenia.[2-4] Recent emerging evidences suggest that small noncoding RNAs known as microRNAs (miRNAs) are involved in pathogenesis and pathological process of the illness.[5-9] miRNAs are a class of ~22 nucleotides noncoding RNAs, which can functionally silence gene in a post-transcriptional manner by complementary base-pairing with target mRNA. Each miRNA can potentially regulate many downstream target genes through intracellular gene silencing machinery.[10,11] The importance of miRNA regulatory network in neuronal development and brain function has been widely studied. Its potential roles have become the focal point in understanding the pathogenesis and development of many neuropsychiatric diseases including schizophrenia. In this review, we summarize the recent development in miRNA regulation and potential functional implications in schizophrenia, with emphasizing on the recent findings of miRNA as novel biological markers and potential therapeutic approach for the disease.

2 | miRNA BIOGENESIS AND FUNCTIONS IN THE CNS

The first miRNA was discovered in Caenorhabditis elegans in 1993.[12] It took 7 years for the second miRNA let-7 to be reported.[13,14] Since 2000, thousands of miRNAs have been identified in animals, plants, virus, as well as in mammalian central nervous system. The functional roles of miRNAs have been widely studied in cell proliferation, differentiation, and apoptosis.[15-19] miRNAs are produced from either their own genes or from introns of coding or noncoding genes. Like the protein-coding genes, miRNAs are transcribed into primary transcripts (pri-miRNA) from genomic DNA by RNA polymerase II or RNA polymerase III. The pri-miRNAs usually possess a 5′ CAP and

a 3′ poly A tail and can be folded into a double-stranded RNA hairpin with a stem-loop structure in the nucleus.[20,21] The stem-loop structure can be recognized by the microprocessor complex containing RNaseIII endonuclease Drosha and nuclear protein DGCR8 (DiGeorge syndrome critical region 8) in vertebrates. Followed the cleavage of 5′ CAP and a 3′ poly A tail by Drosha, the precursor-miRNA (pre-miRNA) with 70~100 nt hairpin shaped is released and exported into the cytoplasm, where the pre-miRNA is cleaved by type III ribonuclease Dicer and resulted in a ~22nt double-stranded RNA duplex: miRNA/miRNA*[22-26] (as shown in Figure 1). During the unwinding process, one strand of the duplex, the mature miRNA, can be loaded into the RNA-induced silencing complex (RISC), whereas the other strand is usually degraded.[27] In addition to miRNA, RISC also contains Dicer, the RNA-binding protein Argonaute (AGO), and the adaptor protein TRBP.[28,29] The miRNA-containing RISC is able to recognize the complementary sequences in the 3′UTR of target mRNAs, consequently results in translational repression or degradation of the target mRNAs.[30] The recognition of the miRNA with its target mRNAs is guided by the initial 2-7 bases of miRNA.[31] Because of the complementary silencing mechanism, one miRNA could target to hundreds or even thousands of mRNAs, whereas different miRNAs can also bind to the same mRNA, which forms a complex miRNAs-genes regulation network.

Approximately, nearly 70% of the known miRNAs are expressed in the mammalian brain.[32-34] Bak et al studied the miRNA expression profiles from 13 distinct areas of the adult mouse brain. They detected 44 miRNAs displaying tissue-specific enrichment, suggesting that CNS miRNAs may be associated with specific function within respective brain region. Although the mature miRNA sequences are conservative between mouse and zebrafish, more than 50% of the identified mouse CNS-enriched miRNAs showed different expression patterns.[18] Recently, Zaits et al presented a comprehensive assessment on spatio-temporal miRNA expression in 18 human donor brains with age ranged from infancy to adolescence. Using RNA sequencing, they reported the expression patterns of miRNAs across both temporal (developmental stages) and spatial dimensions (prefrontal cortex, hippocampus, and cerebellum). The data showed a dramatic shift in miRNA expression shortly after birth. It is noted that most of their target genes for those miRNAs are related to transcriptional regulation, neurodevelopmental processes and common

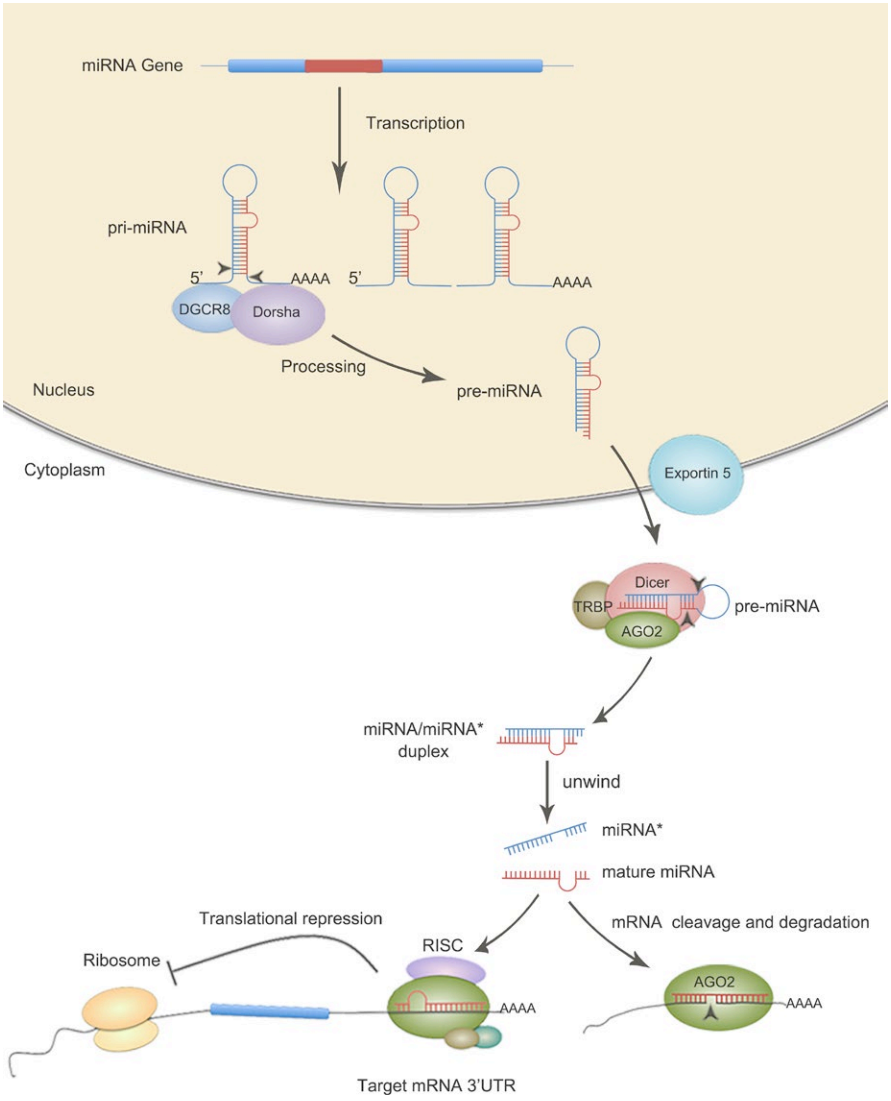

FIGURE 1 Schematic overview of miRNA biogenesis and function miRNAs are transcribed into primary transcripts (pri-miRNA) from genomic DNA by RNA polymerase. PrimiRNAs are cleaved in the nucleus by Drosha to generate miRNA precursor (pre-miRNAs), then exported in the cytoplasm by Exportin-5and further cleaved by Dicer to produce a ~22nt double-stranded RNA duplex: miRNA/miRNA*. One strand of the duplex, the mature miRNA can associate to the RNA-induced silencing complex (RISC) complex and guide translational repression of target mRNAs, whereas the other strand is usually degraded

neurodevelopmental disorders, highlighting the central function of these miRNAs in brain transcription networks.[35] The temporal or brain region-specific manner of miRNA expression may imply the important roles in brain development and neuronal differentiation.[36-40]

The first evidence depicted miRNA role in the CNS was from the Dicer-deficient study in zebrafish in which the animals were found unable to produce matured miRNAs. Dicer mutant zebrafish displayed severe brain developmental abnormality and other malformations including heart. Injection of miR-430, a brain abundant miRNA, was found to rescue many of these defects.[41] In mammalians, Dicer mutant mice died at embryonic day 7.5 before neurulation.[42] Conditional knockout of Dicer in cortex and hippocampus results in the phenotypes of microcephaly, alterations in dendritic branch elaboration and dendritic spine length.[43] Since then, there are numerous reports that revealed the roles of various miRNA in neuronal development and differentiation. The important functional roles of miRNA in neurogenesis, neuroprotection, survival, and pathogenesis of neuropsycho-disorders have been also extensively studied and reviewed.[7,44-49] In addition, alterations of miRNA are reported to be associated with many neuropsychiatric diseases. For instance, selective Dicer depletion in cerebellar Purkinje neurons results in neurodegenerative change, a pathological process similar to progressing neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. More specifically, inactivation of Dicer in the midbrain dopaminergic neurons results in progressive neuron death. This phenotype is significantly rescued by transfection of miRNAs obtained from embryonic mouse midbrain, suggesting that miRNAs play essential roles in midbrain dopamine neuronal differentiation and survival.[50,51] There are other reports depicted various roles of miRNAs on dopaminergic neuron.[52-55] Our group also reported the critical roles of miR-let-7c-5p and miR-3473b in the protective effects of ischemic brain damage via regulating the microglia activation.[56] Given the importance of miRNA in brain functional regulation, it is not surprising that dysregulation of miRNAs may contribute to a pathogenesis and pathological process of many neuropsychiatric disorders. Here, we will further discuss the role of miRNA in schizophrenia.

3 | DYSREGULATION OF mIRNAS IN SCHIZOPHRENIA

3.1 | Altered miRNA expression in schizophrenia

Many studies analyzed the miRNA expression profiles using the postmortem brain samples. Perkins et al examined the miRNA profiles in postmortem prefrontal cortex (PFC) from 13 schizophrenia patients by a customized microarray (miRBase version 7.0). In comparison with 21 psychiatrically unaffected individuals, they found that 15 miRNAs were differentially expressed, in which 14 miRNAs were downregulated and one miRNA (miR-106b) was upregulated in schizophrenia patients.[57] Significant upregulation of miR-181b was also reported in cortical gray matter from the superior temporal gyrus (STG), a brain region involved in the generation of auditory hallucinations of schizophrenia.[58] Two target genes of miR-181b, the calcium sensor gene visinin-like 1 (VSNL1) and the ionotropic glutamate receptor subunit (GRIA2) were identified.[58] The same group further observed a significant schizophrenia-associated increase in global miRNA expression in postmortem tissue in both the STG and the dorsolateral prefrontal cortex (DLPFC).[59] This elevated expression of miRNA is suggested to attribute to an elevation of primary miRNA processing and upregulation of the microprocessor component DGCR8.[59] However, data from Berveridge's study are not consistent with Perkin's report. Some miRNAs such as miR-26b, miR-29c and miR-195 reported to be downregulated in Perkins' study were, however, found to be upregulated in Berveridge's results. Reports on alterations in miRNA expression in schizophrenia are continuously to accumulate. However, the results of miRNA expression profiles are somewhat controversial.[58-67] The discrepancy of miRNA expression data may be explained by differences in sample size, therapeutic protocol, gender factors, and technical application, such as different miRNA extraction methods and miRBase test version. In addition, the temporal expression patterns of miRNAs in human brains could also contribute to the conflict results.[68,69]

To explore the potential role of miRNA as biomarkers, several studies focused on the peripheral blood miRNA expression in schizophrenia patients. Gardiner et al examined miRNA expression in peripheral blood mononuclear cells (PBMCs) from 112 schizophrenia patients and 76 controls. They identified a significant reduction in 83 miRNAs in schizophrenia patients.[70] Lai et al[71] also carried an analysis of genome-wide miRNA expression profile in the mononuclear leukocytes from schizophrenia patients and control groups. Seven miRNAs (upregulated: miR-34a, miR-449a, miR-564, miR-548d, miR-572 miR-652; downregulated: miR-432) associated with negative symptoms and cognitive performance were identified as predictive biomarkers of schizophrenia. Interestingly, they found that the expressions of the seven miRNAs in PBMC were not affected by 2 months of hospitalization, even with a significant improvement of clinical symptoms.[72] It is also noted that the altered expression of hsa-miR-34a and hsa-miR-548d in the blood was not presented in the brain samples.[72] Wei et al[73] also screened the plasma miRNA profiles in a larger sample and identified eight differentially expressed miRNAs (miR-122, miR-130a, miR-130b, miR-193a-3p, miR-193b, miR-502-3p, miR-652, miR-886-5p) in patients with schizophrenia. They also found that the increased levels of miR-130b and miR-193a-3p in patient plasma disappeared after 1 year of treatment with aripiprazole and risperidone and proposed the potential role as biomarkers for prognosis of schizophrenia. In addition, Gallego et al compared the miRNA expression profiles between cerebrospinal fluid (CSF) and the whole blood from schizophrenia patients and healthy controls. However, the miRNA expression levels in CSF and blood were poorly correlated.[74] Although the potential biomarker of miRNAs in schizophrenia patients have been suggested, it is clear that more studies are needed. Indeed, serum miRNAs measurement provides a feasible way for clinical diagnosis and prognosis of schizophrenia including the therapeutic responses.

3.2 | Dysregulation of miRNA biogenesis in schizophrenia

Aberrations in miRNA biogenesis and processing pathway are considered to be involved in the pathological process of schizophrenia. Generation of 22q11.2-deletion mouse was one of the strongest evidence that associated schizophrenia with dysregulated miRNA biogenesis.[75] Microdeletion of human 22q11.2 locus leads to behavioral and cognitive deficits, and a high risk of schizophrenia. Stark et al generated a mouse strain (Df(16)A+/− mice) carrying a hemizygous chromosomal deficiency corresponding to human 22q11.2 microdeletion, and observed schizophrenia-like behaviors in mice. They reported that altered miRNA biogenesis in the brain and found that the dysregulated biogenesis was due to haploinsufficiency of the Dgcr8 gene, which contributes to the abnormal behavior and neuronal deficits in schizophrenia.[75] In further study, the same group found a drastic reduction in miR-185 in Df(16)A+/− mice, which resides within the 22q11.2 locus. The reduction (~70%-80%) of miR-185 in both hippocampus and PFC was more than expected by a hemizygous deletion (~50%) and contributed to the deficits of dendritic and spine development. miR-185 was demonstrated to repress a previously uncharted inhibitor Mirta22 (miRNA target of the 22q11.2microdeletion) which resides in the Golgi apparatus with higher expression in prenatal brain. Reduction in miR-185 expression in the brains of Df(16)A(+/−) mice leads to the sustained derepression of Mirta22 after birth and results in the structural alterations in the hippocampus and cognitive function.[5] Schofield et al[76] examined the Dgcr8+/− mice and found that the reduced expression of Dgcr8 and miRNA emerged over postnatal development during pyramidal neuron maturation rather than in neonatal mice. Altered electrophysiological properties, decreased complexity of basal dendrites, and reduced excitatory synaptic transmission were observed in layer V pyramidal neurons in Dgcr8+/− mice.[76,77] Earls et al reported an age-dependent increase in long-term potentiation in the hippocampus of Dgcr8+/− mice. This increase was attributed to the loss of two miRNAs (miR-25 and miR-185) which target the sarco (endo) plasmic reticulum Ca2+ ATPase (SERCA2). Elevated expression of SERCA2 was found in postmortem sample in PFC and hippocampus of schizophrenia patients.[78] The decreased expressions in Dgcr8 and miR-185 were also confirmed in peripheral leukocytes in individuals with 22q11 deletion syndrome.[79] These findings suggest a pathogenic association between miRNAs and schizophrenia.[78,80]

Dicer is another key gene in miRNA processing pathway. Deletion of Dicer in both zebrafish and mice displays severe defects in brain development.[9] A genome-wide scan for finding copy number variations (CNVs) in schizophrenia identified a de novo duplication in one individual that included DICER1 gene.[81] In a case-control analysis of Chinese population, the SNP (rs3742330) in DICER was reported to be highly associated with schizophrenia risk.[82] The analysis for postmortem brain tissue of DLPFC in schizophrenia patients revealed an upregulation in Dicer expression as well as a global increase in miRNA expression.[59,64] Beveridge et al reported that global miRNA expression was at highest level in early year and declined significantly after adolescence. Dicer and Exportin-5 were also age-dependent but were not correlated with miRNA expression across the lifespan.[83] They proposed that in schizophrenia, neurodevelopment-associated miRNAs remain at a high level after adolescence instead of declining to a lower level in normal subject. The high levels of miRNA expression may cause inappropriate gene silencing that may consequently contribute to abnormal behaviors in schizophrenia.[83] In support, Konopka et al[84] found the enhanced learning and memory in adult mice with tamoxifen-induced deletion of Dicer1 gene. However, when using knockout mouse models, some consideration should be taken into account as pointed out by Rajman.[7] Indeed, Dicer1 has been proved to be essential for cell survival and embryonic development. Dicer1 gene deletion is often associated with massive cell apoptosis and developmental abnormalities,[43,50,85,86] which may complicate the phenotypes and relevance to schizophrenia. On the other hand, not all miRNAs are dependent on Dicer. Some miRNAs are generated through diverse Drosha-independent and Dicer-independent mechanisms.[87-89] Therefore, it is possible that some phenotype observed in Dicer1-deficient mice might be not directly related to the loss of miRNAs, and some functional miRNAs might be missed in these Dicer1 knockout models. It should be bear in mind that the miRNA network is a complicate regulatory system and caution should be taken to interpret functional role of a specific miRNAs underling a phenotype in Dicer knockout model. In this regards, miRNA families or clusters may help to provide more easily interpretable information.[7]

3.3 | miRNA-associated single nucleotide polymorphisms in schizophrenia

Single nucleotide polymorphisms (SNPs) or copy number variations (CNVs) are common DNA sequence variations within populations, which occur more frequently in noncoding region and contribute to human disease susceptibility.[90-92] Using a case-control study, several SNPs of miRNA genes associated with schizophrenia have been reported. SNP rs17578796 in miR-206 showed a significant association with schizophrenia in Scandinavian (Danish and Norwegian) samples.[93] Variant ss178077483 located in the pre-mir-30e was strongly associated with schizophrenia in Han Chinese population (allelic $P = 0.00017$; genotypic $P = 0.00015$).[94] Watanabe et al[95] replicated the association in Japanese population. The expression levels of mature miR-30e in the peripheral leukocyte were significantly higher in schizophrenia patients,[94] consistent with increased expression in the PFC of schizophrenia individuals.[57] Also in Chinese samples, two SNP (hsa-pre-mir-146a rs2910164 G>C and hsa-mir-499 rs3746444 T>C) were genotyped with susceptibility to schizophrenia from 268 patients and 232 controls. Patients carrying CC genotype of rs3746444 were more likely to develop hallucination and lack of motivation. However, there was no statistically significant association between these two SNPs and schizophrenia.[96] Negative association was also observed in SNP rs7289941.[97] Very recently, Yu et al performed a two-stage GWAS of schizophrenia comprising 4384 cases and 5770 controls, followed by independent

replications of 13 single nucleotide polymorphisms in an additional 4339 schizophrenia cases and 7043 controls of Han Chinese ancestry. They confirmed that three loci, at 2p16.1 (rs1051061, in an exon of VRK2), 6p22.1 (rs115070292 in an intron of GABBR1), and 10q24.32 (rs10883795 in an intron of AS3MT; rs10883765 at an intron of ARL3), are significantly associated with schizophrenia. These three loci are known to be involved in the regulation of GABAergic and dopaminergic signaling, cell adhesion molecules, and myelination pathways.[98]

The hairpin structure guides correct miRNA processing, and the 3′UTR of mRNA affects miRNA/mRNA interaction. SNPs in these regions may contribute to the risk of disease. Screening for the mutations in the susceptibility locus (22q11) of schizophrenia in Russian population identified a polymorphism in the 5′-upstream of miR-130b gene region, which contains DNA elements for putative transcription factors. However, genetic analysis did not show statistically significant association of miR-130b variants with schizophrenia.[99] Liu et al[100] applied the MiRSNP database (http://cmbi.bjmu.edu.cn/mirsnp), a collection of human SNPs in predicted miRNA-mRNA-binding sites to GWAS for schizophrenia, and identified seven miRNA-related SNPs. In another study, 803 SNPs from the 3′UTR of 425 schizophrenia-associated genes were analyzed for Gibbs-free energy of miRNA binding in silico. One uncharted SNP (rs3219151 of GABRA6) was reported to be significantly associated with the decreased risk of schizophrenia. Another SNP rs10759 (RGS4) interfered with the miR-124 binding to RGS4, therefore could increase the risk of schizophrenia.[101] Most of the SNP analysis was performed in Chinese Han population. John et al[102] investigated the association of MiRSNPs in candidate genes with schizophrenia in a large population of genetically distinct north Indian cohorts(1017 cases and 1073 controls). They reported that five SNPs were associated with tardive dyskinesia and twelve SNPs were associated with strong schizophrenia genes. Recently, a genome-wide investigation in Canadian patients revealed an enrichment of rare CNVs that overlap miRNAs by excluding the 22q11.2 CNVs, which is known to possess a high susceptibility for schizophrenia.[103] The predicted target genes of the 25 CNV-overlapped miRNA were tended be involved in neurodevelopmental processes.[103] These studies suggest that miRNAs, by targeting schizophrenia risk genes, may contribute to this complex neuropsychiatric disorder.

Recently, a polymorphism (rs1625579) within an intron of primary transcript for miR-137 has attracted lots of attention, which was found to be significantly associated in GWAS of schizophrenia ($P = 1.6 \times 10^{-11}$).[104] Several studies replicated the correlation between miR-137 polymorphisms and schizophrenia samples in Scottish,[105] Canadian,[106] Australian,[107] and Chinese Han population.[108,109] However, negative association results were also reported in some case-control studies.[110,111] In the study of schizophrenia Psychiatric GWAS Consortium, four putative targets gene (CSMD1, C10orf26, CACNA1C, and TCF4) were also reported to have genome-wide significant association with schizophrenia. Luciferase report assay confirmed the interplay between miR-137 and these four target genes,[112] similar results were found for ZNF804A[113] and CALN1.[114] Using bioinformatics resources, several target genes were revealed, including ERBB4, GABRA1, GRIN2A, GRM5, GSK3B, NRG2, and HTR2C. These genes were identified to be involved in synaptic long-term potentiation, a process that may underline mechanism of learning and memory which is impaired in schizophrenia patients.[115] Using functional magnetic resonance imaging scans, van Erp et al[116] reported that the rs1627759 TT (miR-137 locus) is associated with DLPFC hyperactivity, which is a common measure of brain inefficiency. The relationship between rs1625579 genotypes and miR-137 expression was reported by Guella et al[117] They observed lower miR-137 expression levels in the homozygous TT subjects compared to TG and GG subjects in the control group. The reduced miR-137 levels in TT subjects corresponded to increased levels of the miR-137 target gene TCF4.[117] In SH-SY5Y dopaminergic cell line, Strazisar et al[118] also demonstrated that expressing the miR-137 variants resulted in reduction in mature miR-137 expression and lead to the deregulation of gene sets involved in synaptogenesis and neuronal transmission. However, Siegert et al observed gain of function of miR-137 while carrying variants alleles. They found that the increased expression of miR-137 caused the downregulation of the presynaptic target genes such as complexin-1 (Cplx1), Nsf and synaptotagmin-1 (Syt1), and lead to impaired vesicle release. In vivo, miR-137 gain of function resulted in changes in synaptic vesicle pool distribution, impaired induction of mossy fiber long-term potentiation and resulted in defects in hippocampus-dependent learning and memory.[119] All these observations implicate that altered miR-137 may play a critical role in pathophysiology of schizophrenia.

3.4 | miRNA and synaptic plasticity in schizophrenia

Schizophrenia is considered as a complex neurodevelopmental disease. Substantial evidence suggests that dysfunction of several neurotransmitter systems (such as dopamine and glutamate) assemblies the pathophysiological processes of schizophrenia.[54,120] N-methyl-D-aspartate-glutamate (NMDA) receptor is an important regulator in synaptic plasticity. Hypo-function of NMDA receptor signaling could alter the balance of excitation and inhibition in cortical circuits and produces behaviors resembling the symptom of schizophrenia.[121,122] Using a NMDA receptor antagonist dizocilpine, which could rapidly induce schizophrenia-like behaviors, Kocerha et al examined the miRNA expression in different brain regions of mice. They found that mice treated with acute rather than chronic dizocilpine showed a significant decrease in a brain-specific miRNA, miR-219 in the PFC. Decreased miR-219 expression was also observed in hypomorphic GRN1 (NR1) mutant mice. Pretreatment with antipsychotic drugs (haloperidol and clozapine) could prevent dizocilpine-induced reduction of miR-219. One of miR-219 targets was identified as calcium/calmodulin-dependent protein kinase II gamma subunit (CaMKIIγ), a component of the NMDA glutamate receptor signaling cascades. Inhibition of miR-219 in mouse brain reduced the dizocilpine-induced behavioral responses such as hyperlocomotion and stereotypies, suggesting the regulatory role of miR-219 in NMDA receptor function.[123] In support, miR-219 was reported to be significantly

upregulated in the DLPFC of postmortem brain tissue with schizophrenia.[59] In addition, miR-129 was found to participate in the regulation of oligodendrocyte differentiation and myelin maintenance,[124] suggesting the importance of miR-219 in synaptic structure and disease-related function. Zhang et al performed an association analysis for 3 SNPs in hsa-pri-miR-219/132/107 and 6 SNPs in 3′UTR of NMDAR signaling pathway genes (GRIN2A/2B/3A and CAMK2G) in a case-control study of 1041 schizophrenia patients and 953 healthy controls, and confirmed that GRIN2B rs890 was significant associated with schizophrenia.[73]

Brain-derived neurotrophic factor (BDNF) is the most prevalent growth factor in the CNS and plays an essential role in the brain development and neuronal plasticity.[125] Accumulating evidence suggests that dysregulation of BDNF linked to multiple neuropsychiatric disorders.[125,126] Postmortem studies revealed altered BDNF expression level in certain brain regions of schizophrenia patients.[127-130] Mellios et al[60] identified that two miRNAs, miR-30a and miR-195 directly targeted to BDNF 3′UTR and inhibited BDNF expression. They further reported that miR-195 interaction with BDNF could subsequently regulate schizophrenia-related gamma-aminobutyric acid (GABA), that is, GABAergic gene expression, including neuropeptide Y (NPY) and somatostatin.[131] miR-30a-5p was also demonstrated to control alcohol intake by regulating the BDNF signaling pathway.[132]

Cognitive impairment is one of the severe symptoms of schizophrenia.[133] The analysis for the 3′UTR of 242 presynaptic and 304 postsynaptic proteins revealed that 91% of these proteins are predicted miRNA targets.[134] The functional role of miR-132 in learning and memory is suggested to be associated with its regulatory effects on synaptic plasticity. Some behavioral tasks associated with learning and memory were found to rapidly induce miR-132 expression. Knockdown miR-132 in vivo impaired the memory acquisition in trace fear-conditioning paradigm,[135,136] while overexpression of miR-132 in a transgenic mouse model showed increases in neuronal spine density and improvement in novel object recognition.[137] Interestingly, Hansen group reported that mild upregulation of miR-132 enhanced spatial learning of mice. However, a more than three-fold increase in miR-132 expression impaired learning.[138] Double-knockout miR-132/212 impaired the long-term potentiation as well as cognitive function in spatial memory, recognition memory, and in tests of novel object recognition, indicating an important role of miR-132/212 in synaptic function.[139] Compared the transcriptional profile of the hippocampus in respective miR-132 and miR-212 overexpression mouse and miR-132/-212 double-knockout mice, RNA sequencing results revealed that 1138 genes expression were increased in miR-132/-212 deletion mice. Ninety-six of those genes were downregulated in mice overexpressing miR-132. Of the 58 genes that were decreased in overexpressing miR-212 mice, only 4 of them were increased in the double-knockout line.[140] Although miR-132 and miR-212 share a seed sequence, these two miRNAs do not overlap greatly for mRNA targeting genes, suggesting a complex, nonredundant manner in transcriptional profile regulation.

Glial cells play a critical functional rile in brain function. Altered glial functions are known involved in the pathophysiological process in many neuropsychiatric diseases[141,142] including schizophrenia.[143-146] Previous work on miRNA in schizophrenia is focused on neuronal functions, how the glial miRNA contributes to the pathogenesis and development is largely unknown. We recently employed the GFAP-GFP transgenic mice to profile the miRNA in astrocytes in response to acute phencyclidine (PCP) administration. Compared to the saline group, mice treated with PCP showed altered expression of miRNAs (miR-143-3p, miR-212, miR-127-3p, miR-183, miR-298, miR-381-3p, miR-338-3p, miR-let-7d-3p, miR-132-3p, etc.) in astrocytes of PFC (as shown in Table 1). In further study, we explore the functional role of miRNAs with schizophrenia and found the correlation between miR-143-3p dysregulation and PCP-induced behavioral deficits in mice (T. Cao, P. Wang, C. Lu & X. Zhen, unpublished data).

4 | PERSPECTIVE OF miRNA THERAPIES

miRNAs were predicted to regulate 20%-30% of human genes.[147,148] The involvement of miRNAs in many aspects of psychiatric disorder makes them potential candidates as biomarkers or targets for clinical diagnosis and treatment.[6,7,9,149] Although the importance of miRNAs in brain functions has been well-documented, more efforts are required to understand the full extent of miRNA regulatory mechanism and their pathological effects in neuropsychiatric disorders. The first challenge remained is to identify functional miRNAs located in human genome associated with schizophrenia. Project such as the Encyclopedia of DNA Elements project (ENCODE), which aims to map all functional elements in the human genome, has made great progress.[150] Methods of RNA microarray and novel high-throughput technologies such as next-generation sequencing (NGS) provide powerful tools for the analysis of miRNA transcription profiles and their target genes in diseases.[151-154] miRNAs regulate target genes through the base pair interaction of seed region (nucleotide 2-7 of the miRNA) with target mRNA 3′UTR. A sequence of this length will occur with much high frequency in the whole genome; therefore, the prediction of functional miRNA target sites is challenging but will be critical task. Currently, the most comprehensive and wildly applied prediction programs are TargetScan and PicTar. However, two-thirds of their predicted targets appeared nonresponsive to the miRNA.[155] Thus, developing or continuing improvement in experimental tools to understand the miRNA effect on target genes is definitely needed.

Given the abnormal expression of miRNAs in many psychiatric disorders, inhibition or overexpression of miRNAs may be a potential approach in clinical treatment.[156-160] Much effort has been taken to develop high efficiency and nontoxic oligonucleotide mimetics or antisense oligonucleotides to regulate miRNA expression levels. In associated with these efforts, many chemical modifications such as 2′-O-methoxyethyl and locked nucleic acid (LNA) were developed in to enhance the stability of the RNAs.[161-165] Systemic delivery of LNA-antimiR-212 in the liver of African green monkeys leads to a

TABLE 1 MicroRNA sequencing analysis of the mouse prefrontal cortex (PFC) astrocytes in response to acute phencyclidine (PCP) treatment

MATURE miRNA ID	PRE-ACC	MATURE-SEQ	PCP vs Control fold change
PCP vs Control 2.0-fold change Upregulated miRNAs			
mmu-miR-212-5p	MI0000696	ACCUUGGCUCUAGACUGCUUACU	4.06
mmu-miR-127-3p	MI0000154	UCGGAUCCGUCUGAGCUUGGCU	2.90
mmu-miR-183-5p	MI0000225	UAUGGCACUGGUAGAAUUCACU	2.48
mmu-miR-298-5p	MI0000398	GGCAGAGGAGGGCUGUUCUUCCC	2.46
mmu-miR-381-3p	MI0000798	UAUACAAGGGCAAGCUCUCUGU	2.42
mmu-miR-338-3p	MI0000619	UCCAGCAUCAGUGAUUUUGUUG	2.40
mmu-let-7d-3p	MI0000405	CUAUACGACCUGCUGCCUUUCU	2.38
mmu-miR-132-3p	MI0000158	UAACAUGCUACAGCCAUGGUCG	2.22
mmu-miR-130a-3p	MI0000156	CAGUGCAAUGUUAAAAGGGCAU	2.19
mmu-miR-744-5p	MI0004124	UGCGGGGCUAGGGCUAACAGCA	2.17
mmu-miR-330-5p	MI0000607	UCUCUGGGCCUGUGUCUUAGGC	2.13
mmu-miR-335-3p	MI0000817	UUUUUCAUUAUUGCUCCUGACC	2.06
mmu-miR-29c-3p	MI0000577	UAGCACCAUUUGAAAUCGGUUA	2.04
mmu-miR-181a-1-3p	MI0000697	ACCAUCGACCGUUGAUUGUACC	2.04
mmu-miR-872-5p	MI0005549	AAGGUUACUUGUUAGUUCAGG	2.00
PCP vs Control 2.0-fold change Downregulated miRNAs			
mmu-miR-143-3p	MI0000257	UGAGAUGAAGCACUGUAGCUC	0.26
mmu-miR-15a-5p	MI0000564	UAGCAGCACAUAAUGGUUUGUG	0.45
mmu-miR-1968-5p	MI0009965	UGCAGCUGUUAAGGAUGGUGGACU	0.50
mmu-miR-582-3p	MI0006127	UAACCUGUUGAACAACUGAAC	0.50

GFAP-GFP transgenic mouse brains were rapidly removed 30 min after subcutaneous injection with PCP (4 mg/kg) or saline as control. GFP positive astrocytes were sorted by flow cytometry from prefrontal cortex. RNA was extracted from sorted astrocytes and used to prepare the miRNA sequencing libraries. The libraries were captured on Illumina flow cells, amplified in situ as clusters and finally sequenced for 36 cycles on Illumina HiSeq following the manufacturer's instructions. Trimmed reads were alignment to the miRBase pre-miRNAs. miRNA read counts were normalized as tag counts per million miRNA alignments. Differentially expressed miRNAs (PCP vs Control >2.0 fold changes) were presented in the table.

long-lasting and reversible decrease in total plasma cholesterol without any evidence for LNA-associated toxicities.[162] Treatment of hepatitis C virus (HCV)-infected chimpanzees with LNA-antimiR-212 (SPC3649) also leads to long-lasting suppression of HCV viremia, with no evidence of viral resistance or side effects in the animals.[166] The first-in-human study tested a miR-16-based miRNA mimic packaged in TargomiRs-EDVs targeted to EGFR in patients with malignant pleural mesothelioma. The results showed that TargomiRs at a dose of 5×10^9 per week with full dexamethasone prophylaxis were well tolerated and was accompanied by early signs of antitumor activity. This is an open-label study, and a randomized phase 2 study with larger population is needed to confirm the observation.[160]

When exploring such therapy for neurotherapeutics, the major barrier is the blood-brain barrier (BBB) in the CNS. Several approaches for delivering drugs to the CNS have been developed to enhance the capacity of therapeutic molecules to cross the BBB by modifying the drug itself, or by coupling it to a vector.[167] The mediator of exosomes used as a nano-delivery system has several advantages as delivery vehicles.[168,169] Exosomes are the smallest membranous vesicles with homogenous shape and are secreted by diverse mammalian cells. Due to low immunogenicity, remarkable delivering properties and the ability to cross the BBB, exosomes have been considered as efficient delivery mediators for RNA therapy.[168,170] Whether the exosomes can be applied to deliver miRNAs into CNS still need to be tested. Hwang et al have developed a brain-specific nanocarrier, RVG-SSPEI (rabies virus glycoprotein-disulfide linked polyethyleneimine) to successfully deliver miR-124a in mouse brain.[171] Other approaches such as viral delivery systems, chemical modification and conjugation strategies, aptamers, and nanotechnologies have been studied. Although the technological innovations are promising, selective delivery into the CNS remains challenging. Meanwhile, the functional activities of the delivered miRNAs in those systems are difficult to be evaluated. There will be a long way to go for establishing a genuine and practical miRNA treatment in clinical practice.

5 | CONCLUDING REMARKS

Exploring miRNAs biogenesis and function in the CNS demonstrate that miRNAs play essential roles in the pathophysiology of

schizophrenia. The precise profiles of changes in miRNA in schizophrenia patients and its association with the prognosis and therapeutic response remain largely unknown. The future work should focus on identifying specific-disease-related miRNA and understand its precise mechanism in regulating the biological pathway and contribution in pathological process. With the technological progress, targeted delivery of miRNA to CNS may provide a potential novel therapeutic approach for the treatment of psychiatric diseases such as schizophrenia.

ACKNOWLEDGMENTS

This study was supported by grants from the National Science Foundation of China [No. 81773702, 81372688, and 81373382] Supports from the Priority Academic Program Development of the Jiangsu Higher Education Institutes (PAPD) are also appreciated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Cao T, Zhen X-C. Dysregulation of miRNA and its potential therapeutic application in schizophrenia. *CNS Neurosci Ther*. 2018;24:586–597. https://doi.org/10.1111/cns.12840