MicroRNAs suggest a new mechanism for altered brain gene expression in schizophrenia

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Neuropharmacologic and genetic association studies implicate dysregulation of NMDA receptor function in the pathophysiology of schizophrenia and bipolar disorder (1). In this issue of PNAS Kocerha *et al.* (2) suggest a novel molecular mechanism whereby a microRNA (miRNA) regulates signaling downstream from the NMDA receptor at the calcium/calmodulin-dependent protein kinase IIγ subunit (CaMKIIγ). They found that acute treatment of mice with the potent NMDA receptor antagonist dizocilpine resulted in down-regulation of miR-219 in mouse prefrontal cortex. Mice in which the expression of the critical NR1 subunit of the NMDA receptor was reduced by 95% (3) also showed a similar down-regulation of miR-219. Treatment with the antipsychotic drugs haloperidol or clozapine attenuated the hyperactivity and stereotypies caused by dizocilpine and prevented the reduction in miR-219 in the prefrontal cortex. A search of miRNA target databases revealed that the mRNA encoding CaMKIIγ was a possible target for miR-219 (several hundred putative mRNA targets were predicted by sequence complementarity). Treating neuronal-like cultured cells with a modified antisense construct to miR-219 to inactivate it increased the expression of CaMKIIγ levels. Conversely, overexpressing miR-219 in cultured cortical neurons caused a robust reduction in CaMKIIγ levels. Infusing the mouse brain with the antisense construct to miR-219 both attenuated the locomotor and stereotypic behaviors caused by dizocilpine and increased the expression of CaMKIIγ in the prefrontal cortex. Kocerha *et al.* concluded that miR-219 plays an integral role in the behavioral manifestations associated with NMDA receptor hypofunction and thus might be relevant to the "locomotor deficits in acute schizophrenia."

NMDA Receptor Activity and miR-219 Levels

Although these observations are intriguing and novel, there are certain inconsistencies that should be noted. The down-regulation of miR-219 was restricted to the frontal cortex after a single dose of dizocilpine, whereas the pathology of schizophrenia is widespread in the cortex (1). The effect disappeared after chronic treatment with dizocilpine although chronic dizocilpine treatment is considered to be a better model of schizophrenia (4). However, mice with chronically reduced NMDA receptor function caused by decreased NR1 expression did exhibit miR-219 down-regulation in both the frontal cortex and the hippocampus (2). It seems counterintuitive that reduction in miR-219 appears to be responsible for hyperactivity in the acute dizocilpine paradigm but reducing miR-219 levels with antisense infusion reverses dizocilpine-induced hyperactivity. Despite these inconsistencies, it is important to note that the gene for miR-219 implicated by Kocerha *et al.* (2) is located at 6p21, which is a putative susceptibility locus for schizophrenia (5).

miR-219 provides a nexus for 2 risk pathways for serious mental illness.

MicroRNAs Inhibit Translation

miRNAs were first discovered 15 years ago in *Caenorhabditis elegans*. Their role in brain function is only just emerging with more than half of the 200 scientific articles on this topic published in the last 18 months. miRNAs are single-stranded RNAs of ≈22 nt in length (6). miRNAs are generated in a multistep process. The primary transcript (pre-miRNA) is processed within the nucleus to yield a ≈70-nt hairpin, from which the mature miRNA is generated in the cytoplasm by cleavage by the nuclease Dicer. After incorporation into the RNA-induced silencing complex, the miRNA binds to the complementary sequences in the 3′ UTR of the target mRNA. The complex activates endonuclease, which degrades the mRNA or directly blocks translation, both of which prevent the expression of the protein. Several hundred miRNAs have been identified, half of which are expressed predominantly or exclusively in brain.

Because individual miRNAs can have hundreds of potential mRNA targets based on computation of sequence complementarities, it is hypothesized that a specific miRNA might be involved in coordinated regulation of protein expression in functional networks such as are involved in brain development (7) or synapse plasticity (8). Computational complementarity is suggestive evidence of a miRNA targeting of an mRNA but is not dispositive. Direct inhibition of translation must be demonstrated in an appropriate cell system. Aside from CaMKIIγ, miR-219 has a number of potential mRNA targets relevant to schizophrenia and bipolar disorder such as synaptotagmin V (9), netrin (10), and serine hydroxymethyltransferase (11).

CLOCK-BMAL1 Regulates miR-219 Levels

There is another intriguing connection between miR-219 and serious mental illness. The CLOCK–BMAL1 complex binds to the enhancer region of miR-219 and regulates the circadian rhythmic expression of pre-miR-219 (12). Inhibition of miR-219 expression in the suprachiasmatic nuclei by infusion of antisense ("antagomir") into the lateral ventricle lengthens the circadian period. The CLOCK gene has been implicated as a susceptibility gene for bipolar disorder in gene association studies (13). Furthermore, knocking out the CLOCK gene in the mouse produces a mania-like behavioral phenotype (14). Thus, miR-219 provides a nexus for 2 risk pathways for serious mental illness: (*i*) psychosis via hypofunction of NMDA receptors through a downstream effect on CaMKIIγ, and (*ii*) mood instability by disruption of CLOCK–BMAL1 function, which has been implicated in bipolar disorder. Interestingly, the expression of miR-219 is not only regulated by BMAL1, but BMAL1 mRNA is also a target of miR-219.

Micro RNAs and Schizophrenia

Postmortem brain studies of schizophrenia and bipolar disorder have revealed reproducible changes in the expression of functionally-interrelated

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See companion article on page 3507.

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proteins involved in synaptic neurotransmission and development (15). The genes encoding most of these proteins do not appear to be located in proximity to susceptibility loci identified in genetic association studies. miRNAs, which like the unseen puppeteer, are able to manipulate the expression of a number of functionally related genes as observed in these postmortem studies. In this regard, Beveridge *et al.* (16) recently reported that the levels of miR-181b are elevated in the temporal cortex in schizophrenia, and 2 of its targeted mRNAs, which are implicated in synaptic dysfunction in schizophrenia, exhibit reduced expression in the same tissue. Thus, miRNAs represent a novel, but attractive, mechanism for deciphering the subtle, complex, and interrelated alterations in gene expression that characterize the brain in serious mental illness.

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