REVIEW

Small RNA regulation of neural gene expression in response to environmental exposure associated with neuropsychiatric syndromes

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Postmortem molecular analysis of the human brain during development and aging suggests there are epigenetic changes reflecting early life experiences. This includes changes in the expression of non-coding RNAs such as microRNA. These molecules alter the regulation of gene expression and can interact with underlying genetic risk factors, contributing to neurological and neuropsychiatric syndromes such as schizophrenia. Recent evidence suggests that these dynamic and influential molecules play an important role in both brain development and the cellular response to stress. In our recent studies, we investigate the role of microRNA in the brains' response to maternal immune activation and adolescent cannabinoid exposure, alone and in combination, as both have been identified as environmental risk factors for this disorder. We found that combined exposure to significantly altered microRNA expression in the left hemisphere of the entorhinal cortex as compared to the right. These changes were dominated by a large subgroup of microRNA transcribed from a single imprinted locus on chromosome 6q32 that is associated with schizophrenia. These changes correlated with altered gene expression in the combined treatment group, with microRNA-gene interactions predicted to regulate neuronal growth and differentiation; development of specific cortical layers; synaptic plasticity and transmission; axonogenesis; gamma-aminobutyric acid neurotransmitter system; and learning and memory formation. These findings suggested that the interaction of both an early and late environmental insult enhances changes in offspring microRNA expression in the brain with possible outcomes relevant to neurological disorders in adulthood.

Keywords: miRNA; brain, entorhinal cortex; prenatal infection; cannabinoid

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Development of the central nervous system (CNS) is a complex and ongoing process spanning embryogenesis to early adulthood. During this critical period an elaborate network of neural connections is established and maintained via activity-dependent remodelling. The developmental processes that lead to functional synapses and their capacity

to undergo activity-dependent remodelling and synaptic plasticity are complex, regulated by thousands of proteins and involve a combination of genetic and epigenetic influences. In humans the dysregulation of these processes can lead to problems with neural circuitry which can manifest as a range of neurodevelopmental syndromes, such as fragile X mental retardation, and psychiatric disorders, such as schizophrenia and bipolar disorder. Although there is a strong, underlying genetic component, epidemiological studies show that exposure to environmental stress significantly elevates the risk for developing neurodevelopmental disorders such as schizophrenia [1-3]. However, the extent and mechanisms by which the environment produces these effects in the brain remains incompletely understood.

Studies now indicate an emerging role for microRNA (miRNA) in the cellular response to stress, with miRNA activation vital to augment CNS recovery from damage in a controlled manner [4-6]. These short, non-coding sequences are abundant in the human brain and have many diverse and important roles in the CNS [7-9]. MiRNA are recognised to play a critical role in modifying gene expression by repressing translation or inducing mRNA degradation [10]. With each miRNA able to modulate the expression of hundreds of genes, they are predicted to post-transcriptionally regulate ~80% of all protein-coding genes and therefore to be involved in all biological processes. Environmental insults that can affect miRNA expression therefore have the potential to alter numerous processes and affect normal brain development. In fact, studies show that exposure to environmental stressors can not only bring about changes in expression of miRNA involved in the development and function of the CNS, it can also alter the expression of genes involved in the miRNA biogenesis machinery [11-13].

However, in order to understand the role of miRNA in the response to environmental stress it is first necessary to understand their role in gene regulation during neurodevelopment. We recently provided the first global characterization of both miRNA and mRNA expression at various stages in the developing rodent brain. By comparing miRNA and gene expression in the mesencephalon, which develops relatively early, with the telencephalon, which develops later [14], we demonstrated both the temporal and regional specificity of miRNA and their target gene expression during neurodevelopment [15]. Throughout development, 87% of expressed miRNA underwent significant changes, with the highest level of change occurring during early development (embryonic days (E) 12-15). MiRNA expression in the telencephalon was significantly lower than the mesencephalon at E12 consistent with the delayed development of this region. We also observed 32 miRNA that were exclusively expressed in the telencephalon during early brain development (E12) that had predicted functions in neurodevelopmental processes. These findings support the concept that the developing brain is sensitive to environmental factors at specific developmental stages, which can lead to differences in the adult brain as a result of altered developmental processes (reviewed by Dudley *et al.*, 2011 [16]).

To further understand the role of miRNA in the developing brains' response to environmental stress, we examined the impact of an early and late environmental stressor, both alone and in combination, on neural miRNA and gene expression in the entorhinal cortex (EC) [17, 18]. This brain region is located in the temporal lobe and is vital for the mediation of conscious memory [19]. Severe alteration of the EC is associated with several disorders of the human brain, importantly Alzheimer's disease, bipolar disorder, temporal lobe epilepsy and schizophrenia [20-25]. We examined the effects of maternal immune activation (MIA) and adolescent cannabinoid exposure (ACE), both of which have been documented to be strongly associated with an increased risk of developing schizophrenia [3, 26-28] and found that the combination of MIA and ACE induced significant differences in miRNA expression, whereas only a small effect was observed for each treatment alone. Interestingly, this effect occurred predominantly in the left hemisphere (98%), the same hemisphere primarily altered in schizophrenia, and was dominated by a large subgroup of miRNA differentially transcribed from a single imprinted locus on chromosome 6q32 [18]. In humans, the syntenic locus (14q32) encodes a large proportion of miRNAs differentially expressed in schizophrenia [29]. Similarly, alterations in gene expression occurred primarily in the combined MIA-ACE group (99%). MiRNA with altered expression in the combined MIA-ACE group were predicted to have evolutionary conserved interactions with a large proportion of the downregulated genes in this treatment group. MiRNA-gene interactions were identified as highly enriched in the *gamma-aminobutyric acid (GABA) signalling* pathway, s*ynaptic transmission*, *transmission of nerve impulse* and *cell-cell signalling*, processes repeatedly implicated in the pathophysiology of schizophrenia. These genes encode proteins with prominent functions in neuronal growth and differentiation; development of specific cortical layers; synaptic plasticity and transmission; axonogenesis; GABA neurotransmitter system; and learning and memory formation. These changes in gene and miRNA expression corresponded with neuropathological alteration in the entorhinal cortex with significant change in radio ligand binding to the serotonin 5HT1A receptor in the brains of adolescent rats exposed to combined prenatal and postnatal

insult.

These findings indicate that the interaction of both an early and late environmental insult can enhance changes in offspring miRNA expression in the EC that correlate with alterations in gene expression. In response to environmental stress, it is highly likely that miRNA play a major role in the developmental abnormalities that underlie numerous neurological disorders. In particular, abnormalities in the EC may contribute to the aberrant behaviours associated with these disorders, directly affecting cognitive processes that are so often impaired in these conditions. Understanding the dynamics that may mediate a person's predisposition to stress-induced neuropathology has major human health benefits and is an important area of research. Therefore, by linking miRNA to key biological processes related to neuropathology in response to environmental stress, we provide attractive targets for drug design that may offer an alternative to current medications with reduced side effects.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Author contributions

S.L.H. designed experiments, performed the analysis and wrote the manuscript. F.R.W. designed experiments and helped with the analysis. M.J.C. conceived and designed experiments, helped with the analysis and co-wrote the manuscript.

Abbreviations

CNS: central nervous system; miRNA: microRNA; mRNA: messenger RNA; E: embryonic days; EC: entorhinal cortex; MIA: maternal immune activation; ACE: adolescent cannabinoid exposure; GABA: gamma-aminobutyric acid.

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