

A role for miR-19 in the migration of adult-born neurons and schizophrenia

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ABSTRACT

The latest miRNA database (Release 21) annotated 2588 and 1915 miRNAs in the human and mouse genomes, respectively.¹ However, the biological roles of miRNAs *in vivo* remain largely unknown. In particular, the physiological and pathological roles of individual microRNAs in the brain have not been investigated extensively although expression profiles of microRNAs have been reported in many given conditions. In a recent study,² we identified miR-19, which is enriched in adult hippocampal neural progenitor cells (NPCs), as a key regulator for adult hippocampal neurogenesis. miR-19 is an intrinsic factor regulating the migration of newborn neurons by modulating expression level of RAPGEF2. After observing the abnormal expression patterns of miR-19 and RAPGEF2 in NPCs derived from induced pluripotent stem cells of schizophrenic patients, which display aberrant cell migration, we proposed miR-19 as a molecule associated with schizophrenia. The results illustrate that a single microRNA has the potential to impact the functions of the brain. Identifying miRNA-mediated posttranscriptional gene regulation in the brain will expand our understanding of brain development and functions and the etiologies of several brain disorders.

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miR-17~92 in the brain

miR-17~92 is a polycistronic miRNA gene, which is a known oncogene.³ However, recently, it has gained attention as a genetic molecule, linked to dominant hereditary disorders in humans. miR-17~92 is transcribed as a long RNA transcript encoding 6 miRNAs that are classified into 4 different miRNA families: miR-17, miR-18, miR-19 and miR-92⁴ (Fig. 1). miRNAs in the same family include nearly identical sequences that are predicted to suppress almost the same target genes.⁵ While it is unclear which miRNA family results in which phenotypes, variable copy numbers of miR-17~92 cause developmental defects, including impaired brain development and functions.⁶⁻⁸ Microdeletion of the gene results in microcephaly and psychiatric disorders whereas microduplication of the gene causes macrocephaly and autistic traits.

miR-17~92 and its paralogs, miR-106a~363 and miR-106b~25 (miR-17 clusters), are evolutionarily conserved in vertebrates and are expressed broadly from embryonic stem cells (ESCs) to adult cells in diverse tissues, including the brain.⁹⁻¹³ miR-17~92-

deficient mice died shortly after birth, with impaired lung and heart development.¹² While mice lacking other paralogs were indistinguishable from wild type, when the paralogs were ablated simultaneously with miR-17~92, double and triple knockouts resulted in embryonic lethality displaying severe developmental defects and additional abnormalities in particular tissues such as the central nervous system.¹² Conditional knockout of miR-17 clusters in developing brain altered the numbers of oligodendrocytes¹⁴ and reduced the size of the brain,^{15,16} further supporting the functional significance of miR-17 clusters in brain development. In the adult brain, miR-17 clusters, expressed in neural progenitor cells (NPCs), regulate neurogenesis in many ways, including proliferation of NPCs, migration and maturation of newborn neurons.^{2,17-20} Depletion of miR-17~92 in adult hippocampal NPCs reduces neurogenesis and is accompanied by depression and anxiety, illustrating the importance of the gene in brain functions.²⁰

While miRNAs in the miR-17 clusters are under the same transcription unit, the expression level of

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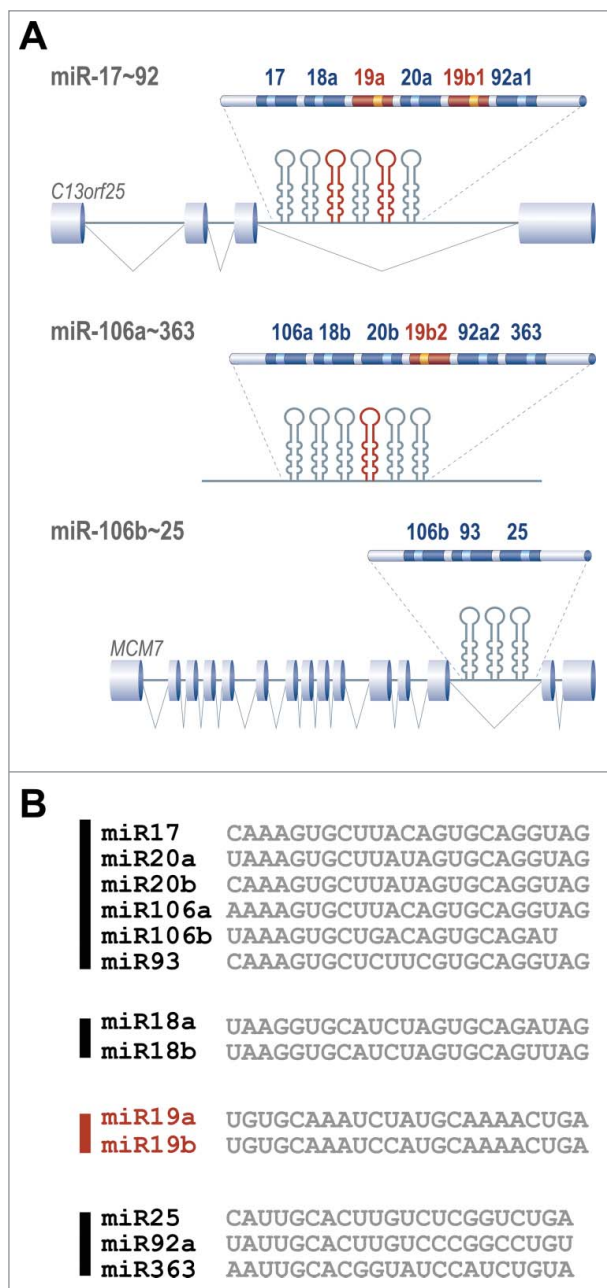


Figure 1. microRNAs in miR-17~92 and its paralogues. (A) Gene structures of miR-17~92 and its paralogues. (B) Four miRNA families generated from the polycistronic genes. This figure is adapted from Mendell (2008).⁵⁸

each miRNA family can be altered independently of other miRNAs in the clusters by posttranscriptional regulation.²¹ The different expression levels and functional contributions of each miRNA family have been shown in the developmental brain;^{14,15,22-24} however, it has been studied less extensively in the adult brain. Abnormal expression of each miRNA family has been reported in adult brains of human patients with Alzheimer disease²⁵ or schizophrenia

(SZ)^{26,27} as well as in a mouse model for amyotrophic lateral sclerosis,²⁸ implying different biological impacts of each miRNA family in the adult brain.

miR-19 in adult hippocampal neurogenesis

Among the polycistronic miRNAs, miR-19 is reduced most dramatically in adult NPCs upon ablation of REST (RE1-silencing transcription factor), a suppressor for neuronal gene expression¹⁸ that accelerates neuronal differentiation of adult NPCs. It is interesting to note that miR-19 is known as a key oncogenic factor in the polycistrons for tumorigenesis,^{29,30} suggesting that a high level of miR-19 may have detrimental effects on diverse cellular processes.

Expression of miR-19 in the adult hippocampus has been confirmed by *in situ* hybridization (ISH).^{2,20} To detect miR-19 at the cellular level *in vivo*, we developed a miR-19 tracer based on a lentivirus that expressed GFP constitutively and RFP in the absence of miR-19. Consistent with the results of the REST knockout study, miR-19 was enriched in adult NPCs and downregulated during neuronal development.² Expression of miR-19 was negligible in ~98% of NEUN+ cells. While NEUN was expressed in a small number of 1-week-old neurons, NEUN expression increased dramatically in ~2-week-old neurons,^{31,32} indicating that miR-19 expression disappeared around 2 weeks after neurons are born.

Next, we investigated biological roles of miR-19 in NPCs in adult hippocampal neurogenesis. To obtain unbiased evidence for biological functions of miR-19 in adult NPCs, genome-wide transcriptome analyses were performed after modulating the expression level of miR-19 in NPCs. It was expected that ~100 genes would change expression in miR-19-overexpressing NPCs compared to control NPCs, making gene ontology (GO) analysis possible. However, very surprisingly, only 10 genes changed their expression in miR-19-overexpressing NPCs.² While the small number of differently expressed genes did not allow GO analysis to be performed, we observed 3 genes that are annotated to be involved in cell migration, leading us to hypothesize that miR-19 controls the migration of newborn neurons. miR-19's role in cell migration is surprising given the findings that several miRNAs such as miR-9 and miR-124 have been shown to be involved in other aspects of adult neurogenesis like proliferation of NPCs,³³⁻³⁶ cell fate determination³⁵⁻³⁷ and differentiation.^{33,34,38-40}

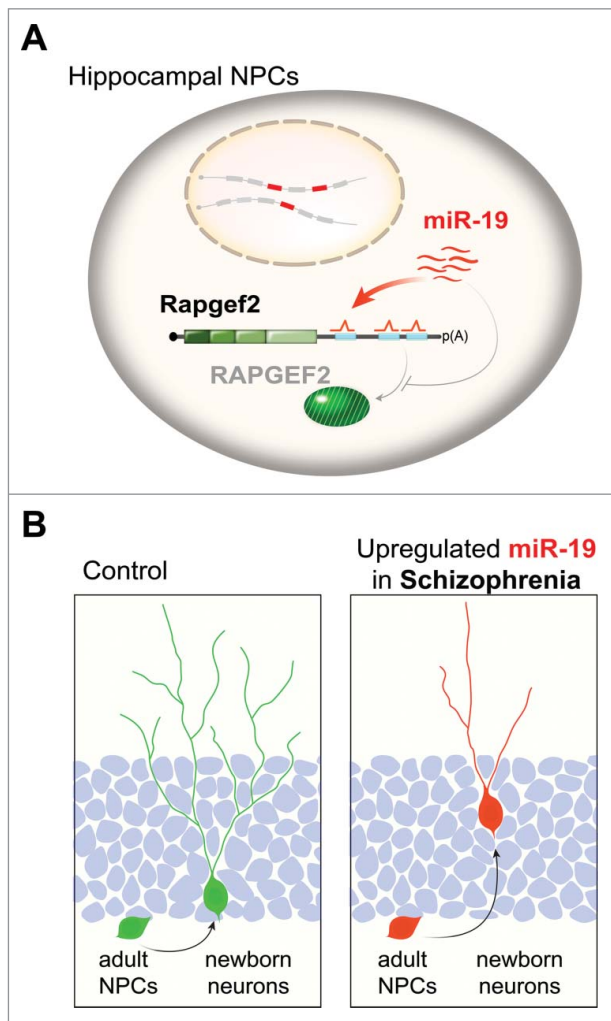


Figure 2. miR-19 functions in adult hippocampal neurogenesis. (A) Expression of miR-19 in adult hippocampal NPCs that modulate RAPGEF2 expression. (B) Extended migration of newborn neurons resulting from upregulated miR-19 and its potential association with schizophrenia.

Overexpressing miR-19 in adult hippocampal NPCs facilitated cell migration and placed newborn neurons in a deeper granule cell layer (GCL)² (Fig. 2). The migration phenotype induced by miR-19 overexpression was detectable even in 8-day-old neurons. Eight days is relevant timing for neuronal migration that occurs in the early stage of neurogenesis. We found that miR-19 works on cell migration by regulating Rapgef2 (Rap guanine exchange factor 2) in adult NPCs. Rapgef2 is a member of Rapgef protein family that governs cell migration by modifying activity of Rap proteins.⁴¹ During early neuronal differentiation, the RAPGEF2 protein level increased, showing an inverse correlation to miR-19 expression level, whereas the Rapgef2 mRNA level did not change. miR-19 suppressed expression of RAPGEF2

protein by binding to Rapgef2 mRNA.² The migration efficiency of newborn neurons increased when Rapgef2 was depleted in adult NPCs, which is similar to the phenotype resulting from overexpression of miR-19 in adult NPCs. Consistent with this finding, the migration of adult-born neurons was attenuated by depletion of miR-19 or overexpression of RAPGEF2 in NPCs.

Under physiological conditions, adult-born neurons migrate very short distances to the GCL and stay in the inner GCL. Therefore, the extended migration of newborn neurons resulting from overexpressing miR-19 in NPCs we observed was unexpected. A comparable migration phenotype has been described in a few other studies manipulating genes involved in neuropsychiatric disorders, such as Pten (phosphatase and tensin homologous) in autism spectrum disorders^{42,43} and Disc1 (disrupted in schizophrenia 1),^{44,45} Plcb1,^{46,47} and Nr3c1 (also known as glucocorticoid receptor)^{48,49} in SZ, suggesting that miR-19 is likely related to neuropsychiatric disorders.

Implications of miR-19 in SZ

Abnormal expression of miR-19 in the brains of SZ patients has been reported in a postmortem study.²⁶ Moreover, copy number variations encompassing *RAPGEF2*, a major target of miR-19 in adult hippocampal NPCs that we identified, has been found in a familial SZ study.⁵⁰ Aberrant cell migration has also been displayed in human NPCs that were derived from SZ patients through reprogramming.⁵¹ Abnormal cell migration was also observed in our study by modulating miR-19 in adult hippocampal NPCs. All these findings indicate a potential link between miR-19 and SZ.

By utilizing human hippocampal NPCs, derived from induced pluripotent stem cells of SZ patients (SZ-NPCs) and relative controls (control-NPCs),⁵² we found that miR-19 was upregulated in SZ-NPCs compared to control-NPCs.² This finding is consistent with a previous study reporting an increase in miR-19 in the postmortem brain of SZ patients.²⁶ In addition, as miR-19-mediated RAPGEF2 regulation is conserved in human ESC-derived hippocampal NPCs, SZ-NPCs express less RAPGEF2 protein than control-NPCs,² demonstrating the significance of miR-19 in operating the migration of newborn neurons that may be linked to the etiology of SZ.

Depression resulting from miR-17~92 knockout in adult NPCs²⁰ is a frequently occurring symptom in SZ patients.⁵³ Epilepsy, which often results in mis-localized adult-born neurons in the GCL, is a known risk factor for SZ.⁵⁴⁻⁵⁶ However, it would be necessary to investigate how the aberrant migration of newborn neurons resulting from miR-19 contributes to SZ pathogenesis specifically.

Conclusion

While genome-wide association studies have revealed genetic loci that are associated with SZ, genetic factors that increase the risk for this complex disease remain largely unknown. The abnormal expression of miR-19 and RAPGEF2 in SZ-NPCs shown in our study² was independent of genomic DNA mutation. Genomic DNA of SZ patients did not have mutations on either *MIR19* or *RAPGEF2*,⁵⁷ indicating that investigating posttranscriptional gene regulation is valuable to elucidate the molecular mechanisms underlying brain disorders. Overall, our work shows that non-biased reverse genetic study of a microRNA can be a desirable strategy to find essential protein coding genes influencing brain development and their physiological functions, as well as their associated diseases in the brain.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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