REVIEW

Emerging roles for miRNA-based post-transcriptional regulation in neuronal morphogenesis and neurodevelopmental disorders

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Post-transcriptional regulation of gene expression is required for multiple aspects of neuronal development and function in the central nervous system. A sub-class of small non-coding RNA, called microRNAs (miRNAs), is emerging as key modulators of post-transcriptional gene regulation in numerous tissues, including the nervous system. Recent evidence has revealed a widespread role for miRNAs in various aspects of neuronal morphogenesis, including axogenesis, dendritogenesis, and synapse formation. Furthermore, dysregulation or altered expression of miRNAs has been associated with the pathogenesis of neurodevelopmental and psychiatric disorders. Here, we highlight recent advances in the study of miRNA-based regulation of neuronal development and their implications in neurological disorders.

Keywords: miRNA; neuronal development; neurodevelopmental disorders; psychiatric diseases; Post-transcriptional gene regulation

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Introduction

Proper formation and maintenance of neuronal morphology is critical for normal brain function. The size and shape of neurons establish the functional neural circuits and neuronal connectivity. Although various proteins/factors, such as transcription factors, cytoskeletal elements, and components of various signaling pathways have been identified as contributors to neuronal morphogenesis, recent evidence has implicated microRNAs (miRNAs) as another important contributor to the regulation of neuronal morphology.

miRNAs are a novel class of small non-coding RNAs containing 19–24 nucleotides. They act as post-transcriptional regulators of gene expression by binding to the 3' untranslated region (3'UTR) of their target messenger RNAs (mRNAs) [1, 2]. miRNA genes are transcribed as primary-miRNA (pri-miRNA) by RNA-polymerase Ⅱ. The primary transcript is then processed

by the RNase Ⅲ enzyme Drosha in the nucleus to generate a precursor-miRNA (pre-miRNA). The pre-miRNA is subsequently translocated to the cytoplasm by an exportin-5 dependent mechanism and is then further processed into mature miRNA by Dicer. The mature miRNAs are then loaded into the RNA-induced silencing complex (RISC), a ribonucleoprotein complex that is composed of the human immunodeficiency virus transactivating response RNA-binding protein (TRBP), argonaute 2 (Ago2), and Dicer, and the resultant complex incorporate target mRNA to negatively regulate gene expression by inhibiting translation or promoting degradation of mRNA [3].

miRNAs have been shown to regulate numerous biological processes such as cell proliferation, differentiation, growth, and apoptosis [4-7]. Accumulating evidence has unveiled important roles of miRNAs in the regulation of brain development and differentiation of neural cells [8-10]. Here, we review recent advances in the understanding of the biological roles of miRNAs in neuronal development and also discuss evidence suggesting the association of these miRNAs with neurodevelopmental diseases including psychiatric disorders.

miRNAs in axonal development

Axons relay information to other neurons through chemical signals. Thus, functional neural circuits require appropriate axogenesis. The existence of mRNA and its post-transcriptional regulation in developing axons is manifesting as a crucial molecular mechanism underlying axonal development. Recent studies have also shown that miRNAs are present in axons and contribute to axonal development.

Several brain-enriched and neuron-specific miRNAs have been identified. Among these miRNAs, the brain-enriched miR-9 has been well studied. A recent study showed that miR-9 is expressed in post-mitotic neurons and is detected in the axons of primary cortical neurons. Overexpression of miR-9 decreases axonal length and inhibition of endogenous miR-9 has the opposite effect, indicating that miR-9 negatively regulates axonal elongation. It has been reported that these effects are emanated through the regulation of miR-9's target; that is, microtubule-associated protein 1b (Map1b), an important protein for microtubule stability [11].

One of the most abundant miRNAs expressed in the vertebrate central nervous system (CNS) is miR-124. The expression of miR-124 is increased during brain developmental events associated with axonal elaboration [12]. An elegant study by Sanuki *et al*. showed that miR-124 regulates axon development by inhibiting *LIM/homeobox protein 2* (*Lhx2*) expression *in vivo* [13]. Another report demonstrated that miR-124 controls axon growth by targeting mRNA of small GTPase RhoG [14].

Zhang *et al*. reported that the components of the miR-17-92 cluster are detected in distal axons of cultured cortical neurons. Increased expression levels of this cluster promoted axonal development, whereas the blocking of miR-19a, one component of the cluster, inhibited axon formation [15].

Recent work identified miR-132 as a positive regulator of axon development of the mouse dorsal root ganglion (DRG). Mechanistically, miR-132 was shown to modulate the expression of Ras GTPase activator Rasa1 [16].

miRNAs in dendrite development

As dendrites are the site of most synaptic contacts, adequate growth and branching of dendrites are important for neural circuitry function. Recent findings have provided evidence that miRNAs participate in the regulation of dendrite development. In this section, we review recent progress in uncovering the miRNA-mediating molecular mechanisms controlling dendrite morphogenesis.

An early study by Smrt *et al*. suggested that brain-enriched miRNA, specifically miR-137, plays a key role in modulating dendrite development. Overexpression of miR-137 negatively controls dendritic morphogenesis, whereas inhibition of miR-137 function had the opposite effect. This effect of miR-137 on dendritic development is mediated by the regulation of Mind bomb one (Mib1), which is a ubiquitin ligase known to be important in neurodevelopment [17]. Another study demonstrated the positive role of miR-134 in dendrite development. miR-134 is a component of miR-379-410, a large cluster of brain-specific miRNAs. This miR-134 promotes dendritic morphogenesis by inhibiting translation of the mRNA encoding the translational repressor Pumilio2, a RNA-binding protein known to regulate dendrite morphogenesis [18]. A genetic experiment demonstrated that miR-132 regulates dendrite maturation of newborn neurons in the adult hippocampus, possibly via modulating the GTPase-activating protein p250GAP [19]. In addition to playing a role in axon development, miR-9 has also been shown to regulate dendritic growth. Inactivation of miR-9 leads to impairment in dendritic development *in vivo* through downregulation of the transcriptional repressor RE1 silencing transcription factor (REST) [20].

Recently, we demonstrated miR-214 had a role in the regulation of dendritic development [21]. We showed that overexpression of miR-214 promotes dendritic growth and complexity, whereas blocking of endogenous miR-214-3p, one of the mature forms of miR-214, suppresses dendritic development. Our study also demonstrated that miR-214-3p targets the conserved 3'-UTR of *quaking* (*Qki*), which is a suggested gene implicated in schizophrenia.

miRNAs in synaptic formation

Several lines of evidence have uncovered that miRNAs act as important regulators of synaptic morphological dynamics and plasticity. Therefore, miRNAs are thought to underlie higher brain functions such as learning and memory [22].

It has been shown that many miRNAs function as negative regulators of synaptic formations. For example, miR-134 was the first reported miRNA that regulates synaptic formation. Overexpression of miR-134 in cultured neurons decreased the size of dendritic spines through translational repression of *Lim-domain containing protein kinase 1* (*LimK1*), a regulator of actin polymerization [23]. miR-34a inhibits synaptic function by targeting mRNAs of the synaptic components synaptotagmin-1 and syntaxin-1A [24]. miR-138, a brain-enriched miRNA, is localized in the dendrite and negatively regulates the size of dendritic spines through the regulation of acyl protein thioesterase 1 (APT1), an enzyme controlling the palmitoylation status of multiple proteins that are known to function at the synapse [25].

Positive regulation of synapse development by miRNAs have also been reported. miR-125b and miR-132 were shown to be associated with fragile X mental retardation protein (FMRP). Increased expression of miR-125b induced longer and thinner processes of cultured hippocampal neurons, whereas miR-132 overexpression led to stubby and mushroom-shaped spine formations [26]. In this work, miR-125b was shown to repress expression of its target, NMDA receptor subunit NR2A, along with FMRP and argonaute 1. Another study supported the positive role of miR-132 on synapse development *in vitro* and *in vivo*, showing that miR-132 represses Rho GTPase activating protein p250GAP expression [27]. miR-132 was also found to target the mRNA encoding the methyl CpG-binding protein 2 (MeCP2), a regulator of neuronal morphology and synaptic formation [28, 29]. Furthermore, we have recently shown that MeCP2 promotes the processing of miR-199a as a component of the microprocessor Drosha complex, and that miR-199a promotes excitatory synaptic transmission and density through targeting mTOR signal negative regulators in the cultured hippocampal neurons [30].

miRNAs in neurodevelopmental disorders

Accumulating studies have suggested that impaired post-transcriptional regulation caused by miRNA dysregulation may contribute to defective neuronal function and plasticity in neurological diseases including neurodevelopmental disorders [31]. miRNAs have been implicated in the pathophysiology of neurodevelopmental and psychiatric disorders [32]. Here, we focus on two miRNAs; i.e., miR-137 and miR-199a, which have been shown to contribute functionally to the pathogenesis of diseases.

Recent genome-wide association study (GWAS) in schizophrenia showed that rs1625579, which is found within the putative primary transcript for miR-137, is associated with an increased risk of schizophrenia [33]. Other groups have also confirmed this association [34, 35]. Moreover, functional MRI (fMRI) studies have demonstrated that variation in miR-137 specifically influences the activity of the posterior right medial frontal gyrus during a cognitive task and functional connectivity of the front-amygdala and dorsolateral prefrontal-hippocampus in emotional tasks [36, 37]. Another study has also shown that increased miR-137 expression levels lead to the downregulation of presynaptic target genes such as *complexin-1* (*Cplx1*) and *synaptotagmin-1* (*Syt1*) *in vitro* and *in vivo*, causing impairments of synaptic vesicle trafficking and alterations in synaptic plasticity [38].

In addition to these findings regarding schizophrenia, we demonstrated a role for miR-199a in the pathogenesis of Rett syndrome (RTT) [30]. RTT is a severe progressive neurodevelopmental disorder caused by *MECP2* mutations. As mentioned above, we have shown that MeCP2 facilitates the post-transcriptional processing of miR-199a as a component of the Drosha complex and miR-199a positively controls mTOR signaling, which is associated with a variety of neurodevelopmental disorders [39], by targeting mRNAs for inhibitors of mTOR signaling such as *Pde4d*, *Sirt1* and *Hif1α*. Genetic deletion of miR-199a was shown to recapitulate numerous RTT phenotypes of MeCP2-knockout mice. Dysregulation of miR-199a expression at a post-transcriptional level was also found in the brain of patients with RTT. These findings suggest that miR-199a, a MeCP2 downstream miRNA, contributes to RTT pathophysiology [30].

Conclusions

We summarized the above-introduced functions of miRNAs in this review in Table 1. Although functional

Table 1. miRNAs involved in neuronal morphogenesis

Function	Effect	miRNAs	Targets	Reference
Axon development	Negative	miR-9	Map1b	[11]
	Positive	miR-124	Lhx2, RhoG	[13, 14]
		miR-19a	PTEN	[15]
		miR-132	Rasa1	[16]
Dendrite development	Negative	miR-137	Mib1	[17]
	Positive	miR-134	Pumilio2	[18]
		miR-132	p250GAP	[19]
		miR-9	REST	[20]
		miR-214	Qki	[21]
Synapse formation	Negative	miR-134	LimK1	[23]
		miR-34a	Synaptotagmin-1, Syntaxin-1A	[24]
		miR-138	APT1	[25]
	Positive	miR-125b	NR2A	[26]
		miR-132	p250GAP, MeCP2	[27-29]
		miR-199a	PDE4D, SIRT1, HIF1a	[30]

characterization of miRNAs in the mammalian neuronal system is still in its infancy, the accumulating evidence described in this review suggests that miRNAs function as key modulators of neuronal morphogenesis and pathological conditions in neurodevelopmental diseases. Considering the facts that many miRNAs are brain-enriched or even specific [40] and that individual miRNA can target multiple genes [41], miRNAs can be considered as a group of molecules dominating the complex process of neuronal development and brain functions. Future studies are required to identify further functional targets of each miRNA and the downstream gene networks that are associated with neuronal functions. Numerous studies have revealed that many miRNA expression levels are altered in a variety of neurodevelopmental disorders [42, 43]. However, currently, functional studies of the miRNAs *in vivo* are still limited. Thus, we expect that elucidating the roles of these miRNAs help to further understand the mechanisms of brain development and pathogenesis of neurodevelopmental disorders, opening new avenues for designing therapeutic strategies that target the miRNA-mediated pathway in the CNS.

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Conflicting interests

The authors have declared that no conflict of interests exist.

Author's contributions

Conceptualization, K.T., H.N., K.I. and K.N.; Writing-Original Draft, K.T. and K.N.; Writing-Review & Editing, K.T., H.N., K.I. and K.N.

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