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Review

MicroRNAs tune cerebral cortical neurogenesis

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MicroRNAs (miRNAs) are non-coding RNAs that promote post-transcriptional silencing of genes involved in a wide range of developmental and pathological processes. It is estimated that most protein-coding genes harbor miRNA recognition sequences in their 3′ untranslated region and are thus putative targets. While functions of miRNAs have been extensively characterized in various tissues, their multiple contributions to cerebral cortical development are just beginning to be unveiled. This review aims to outline the evidence collected to date demonstrating a role for miRNAs in cerebral corticogenesis with a particular emphasis on pathways that control the birth and maturation of functional excitatory projection neurons.
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Facts

- MicroRNAs are enriched in the nervous system and some show a dynamic expression that correlates with important milestones of cerebral cortex development.
- Mouse embryos harbor cortical malformations upon Dicer deletion.
- MicroRNAs underlie cortical development by repressing multiple target genes, some being intricately interconnected.

Open Questions

- What are the upstream mechanisms that drive the dynamic expression of microRNAs in cerebral corticogenesis?
- Are microRNAs directly controlling the migration of postmitotic projection neurons?
- A single miRNA can target hundreds of predicted mRNAs, thus more need to be done to define the full spectrum of genes regulated by miRNAs in cortical neurogenesis.
- What is the exact contribution of miRNAs to neuropathological conditions that affect the development of the cortex and its plasticity during adulthood?

The cerebral cortex (Cx) is the most complex structure of the brain and comprises up to six horizontal layers of neurons (Figure 1). Neurons that belong to the same cortical layer share characteristic morphology, dendrite arborization complexity and axonal projection pattern. In addition, cortical neurons are regionally organized into specific areas that underlie cognitive, motor and perceptual abilities.[1] Most cortical neurons are glutamatergic projection neurons that are born in germinal compartment of dorsal telencephalon. They migrate a short distance along radial glia fibers to settle in dedicated cortical layers. They send axonal projections to distant cortical or subcortical targets. The second class of neurons includes GABAergic interneurons that arise from the ventral telencephalon and travel along multiple tangential paths to reach the cortical wall. They incorporate local neuronal networks where they modulate neuronal excitability.[2] Generation of cortical neurons relies on the completion of cell cycle exit, cell migration and neuronal differentiation. These processes are orchestrated by interplay between extracellular and intracellular signaling cues that ultimately converge on cytoskeleton to support morphological remodelings.[3] Disruption of these cellular processes can result in cortical malformations that are associated with some psychiatric or neurological disorders.[4–8] Thus, it is important to improve our understanding of the molecular pathways that control cerebral Cx development.

MicroRNAs (miRNAs) are abundant short-lived double-strand RNAs of ~20 to 25 nucleotides that are derived from endogenous short-hairpin transcripts. They contribute to various developmental processes in eukaryotes. Indeed, they act as post-transcriptional regulators and thus introduce an additional level of intricacy to gene regulation in neurogenesis.[9–11] Recent data obtained by several groups support a major role for miRNAs in fine-tuning signaling pathways that control the concurrent steps of corticogenesis.[12–18] Slight modifications of their expression have been associated with a range of neurological disorders,[19–26] some affecting cerebral

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Abbreviations: Ac, anterior commissure; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; CC, corpus callosum; Chrdl-1, chordin-like 1; CP, cortical plate; CR, Cajal–Retzius; Cx, cortex; DCX, doublecortin; DDR, DNA-damage response; F/Hc, fornix/hippocampal commissure; IZ, intermediate zone; LSD1, lysine-specific demethylase 1; mEPSC, miniature excitatory postsynaptic currents; Mib1, mind bomb one; miRNAs, microRNAs; MZ, marginal zone; NEPCs, neuroepithelial cells; NSC, neural stem cell; PP, preplate; Sirt1, Sirtuin 1; SP, subplate; SVZ, subventricular zone; UPF1, regulator of nonsense transcripts homolog; VZ, ventricular zone; WT, wild type
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cortical activity.[27–29] The processing steps and core components of the pathway that control miRNA biogenesis are now well characterized. It starts with the RNAse III enzyme Drosha and its binding partner DGCR8/Pasha that cleave primary miRNAs in the nucleus to generate pre-miRNAs, stem-loop precursors of 70 nucleotides.[30] Pre-miRNAs are then exported to the cytosol by the exportin 5 and further processed into mature double-strand miRNAs by the RNAse III enzyme Dicer.[31] Duplexes are then unwound, and one strand is incorporated into a silencing complex (RISC) and guided to its target messenger. This matches miRNAs with their specific seed sequence on the 3′-untranslated region of target mRNAs.[32,33] As such miRNAs can target hundreds of predicted messengers, resulting in their degradation by endoribonucleolytic cleaveage, deadenylation and decapping, or translational inhibition.[9,34,35] There is no dispute that the canonical pathway is critical for maturation of miRNAs, but Drosha- and Dicer-independent biogenesis mechanisms have recently emerged and may account for maintenance of subclasses of active miRNAs in core biogenesis mutants.[36]

Several miRNAs are abundant in the developing cerebral Cx and some show a dynamic expression that correlates with important developmental milestones of the Cx. Mice null for *Dicer* are embryonic lethal.[31] Thus, to bypass early embryonic lethality and analyze miRNA's functions in cerebral cortical development, four conditional mouse lines carrying a deletion of Dicer in the telencephalon have been established. Experiments performed with these genetic models revealed critical roles for Dicer in cortical neurogenesis.[12,13,16,17,37] While it has been generally reasonable to infer that the phenotype of these mice resulted from loss of mature miRNAs, functional connections to individual miRNA were often correlative. Therefore, acute modulation of single miRNA expression was performed *in vivo* or *in vitro* to assign specific functions and targets to these molecules.[38–40] This review summarizes and discusses accumulating evidences that miRNAs are required to ensure proper neurogenesis, differentiation and maintenance of cortical projection neurons. We entertain the hypothesis that RNA interference is a critical process to cortical development and later plasticity, and that its disruption at any developmental steps could further underlie onset of complex human neurological disorders.

miRNAs Promote Survival, Proliferation and Specification of Cortical Progenitors

Dicer controls the homeostasis of cortical progenitors. The cerebral Cx develops from the dorsal telencephalon, which comprises distinct types of progenitors located around the ventricles. Before the onset of neurogenesis, the presumptive Cx is almost exclusively composed of neuroepithelial cells (NEPCs) that proliferate actively and self-renew by symmetric divisions (Figure 1).[41] When neurogenesis

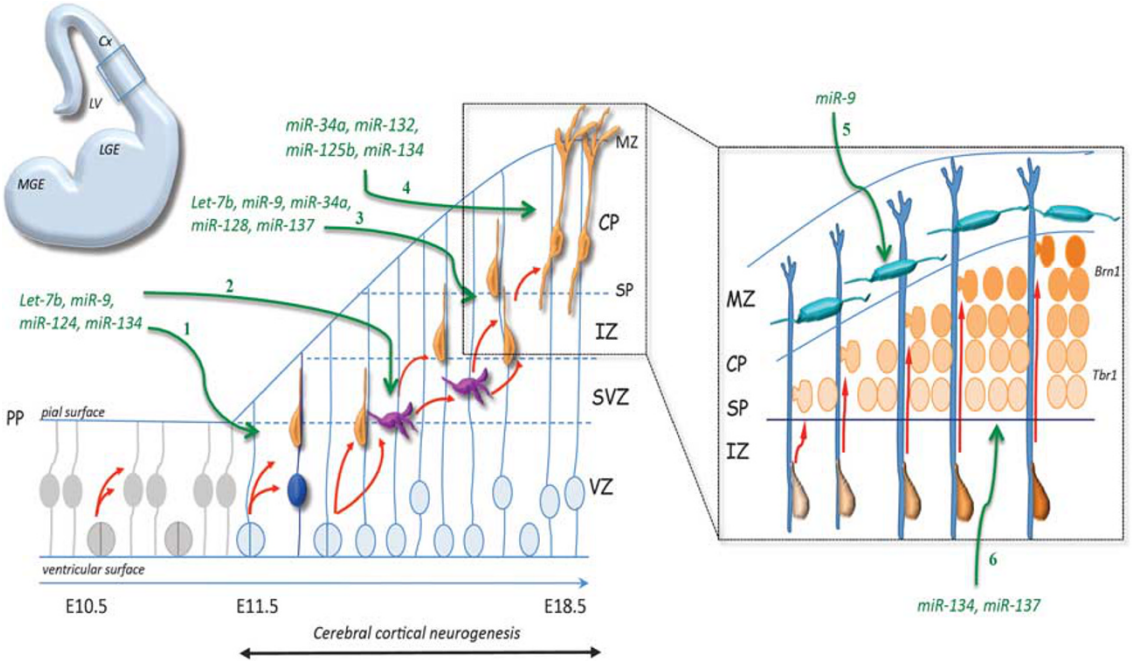


Figure 1 miRNA regulation of cerebral cortical neurogenesis. Scheme representing a part of the cortical wall corresponding to the area boxed in half brain coronal section (upper left). Before onset of neurogenesis (E10.5), symmetrical divisions increase the pool of NEPCs (light gray). Neurogenesis in the Cx starts at E11.5, when some Pax6-positive radial glial cells (blue) divide asymmetrically in the VZ. These cells self-renew (dark blue) and generate neurons in the SVZ either directly (orange) or indirectly through Tbr2-positive basal progenitors (pink). These concurrent steps are regulated by a set of miRNAs: Let-7b, miR-9, miR-124 and miR-134 (1, 2). Basal progenitors can sometime divide asymmetrically to self-renew but also divide symmetrically to give birth to two neurons. Differentiation of projection neurons that migrate through the intermediate zone (IZ) and integrate the CP requires Let-7b, miR-9, miR-34a and miR-137 (3). Terminal differentiation, including dendritogenesis and synapse formation, involves the expression of miR-34a, mir-132, mir-125b and miR-134 (4). The inset (right) shows the inside-out organization of cortical layers. Successive waves of postmitotic neurons are generated (graded orange), and these neurons migrate along radial glial cells (blue) through the IZ to reach the CP. Migration of neurons into the CP is regulated by miR-134 and miR-137 (6). The CP is builded in an inside-out manner and neurons stop their migration under the MZ where Cajal–Retzius cells (CR) (blue turquoise) are located. The generation of CR is controlled by miR-9 (5). MGE, medial ganglionic eminence, LGE, lateral ganglionic eminence, LV, lateral ventricle

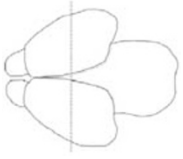
starts in the Cx (E11.5, in mouse), these cells become Pax6-expressing radial glia (also named apical progenitors).[42] Their conversion includes growth of a radial process between pial and ventricular surfaces of the telencephalic wall.[43] Radial glial cells progressively divide asymmetrically to self-renew and give birth to neurons, either directly or indirectly through generation of Tbr2-positive intermediate (basal) progenitors.[44–46] Intermediate progenitors settle in the subventricular zone (SVZ) and divide symmetrically to generate either postmitotic neurons that migrate along radial glia fibers to reach the cortical plate (CP) or, more rarely, additional pairs of progenitor cells [46] (Figure 1).

Recent works described the contribution of Dicer and mature miRNAs to cortical development. Conditional knockout of Dicer in the Cx was achieved after breeding Dicer:lox/lox mice with various forebrain Cre-driver mouse strains, including Nestin:Cre, Emx1:Cre or FoxG1:Cre. However, low amounts of miRNAs remained detectable at different developmental stages, depending on the mouse strain utilized. In all cases, conditional removal of Dicer led to significant reduction in thickness and/or size of the presumptive Cx, which likely resulted from combined defects of proliferation and survival of cortical progenitors and their progeny (Table 1).

De Pietri Tonneli *et al.* described a progressive cell apoptosis throughout the cortical wall of Emx1:Cre;Dicerlox/lox mouse embryos that started at embryonic day (E) 12.5. Indeed, it was recently shown that cells accumulated DNA double-strand breaks and activated the $p19^{ARF}$-p53 signaling pathway upon Dicer inhibition *in vitro*,[47,48] a process that can ultimately lead to apoptosis.[49] This is not surprising as miRNA-mediated gene silencing is an integral part of the DNA-damage response (DDR) pathway and miRNAs target several players of the DDR signal–transduction network.[50–54] Impairment of the DDR pathway is likely to occur upon Dicer removal in the Cx as suggested by the work performed in our laboratory (M-L Volvert and L Nguyen; unpublished data). Numbers of apical and basal progenitors were reduced from mid-corticogenesis (E14.5) in Emx1:Cre;Dicerlox/lox embryonic Cx. The poor survival of basal progenitors likely accounts for the reduced number of upper-layer neurons upon Dicer removal. In addition, the remaining ones were misplaced and mixed with deep-layer neurons.[13] Complementary data obtained by others suggested the precious differentiation of cortical progenitors into deep-layer neurons in the same transgenic mouse line,[16] thus supporting a complex phenotype that arise from multiple cellular defects after removal of Dicer in Emx1-expressing cells.

Genetic removal of Dicer with Nestin:Cre mouse strain led to milder and latter cerebral cortical defects. Although the expression of Cre started around the same developmental stage (Table 1), the thickness of cortical wall was only reduced at late developmental stages as compared with embryos from Emx1:Cre-driver mouse line. Moreover, cortical progenitors and their Tbr1-positive cell progeny remained unaffected until E15.5. Indeed, measurable defects started at E18.5, including elevated cell death of cortical progenitors, misplacement of Tbr1 positives upper-layer neurons as well as an overall delayed differentiation of projection neurons, as attested by strong reduction in NeuN expression in the CP.[16] Differences observed between Nestin:Cre and Emx1:Cre mouse cortices arise from distinct Cre-induced recombination efficacy of

Table 1 Dicer conditional knockout mouse models

	Emx1:Cre; Dicer: loxP/loxP (a, b)	**Nestin:Cre; Dicer: loxP/loxP (b)**	**Foxg1:Cre; Dicer: loxP/loxP (c, d)**	**CamKII:Cre; Dicer: loxP/loxP (e)**
					
Timing and location of Cre expression	From E10.5, in most cells of the dorsal telencephalon	From E10.5, in forebrain stem cells and progenitors	From E9.5, in most forebrain cells	From E15.5, in postmitotic neurons of the cortex and the hippocampus
Brain size and cortical thickness	Reduced cortical thickness from E13.5 and enlarged ventricles	Reduced cortical thickness from E18.5 and enlarged ventricles	Reduced size of the forebrain and reduced cortical thickness from E13.5	Microcephaly, enlarged ventricle size
Cell survival	Increased cell death from E12.5	Increased cell death from E18.5	Increased cell death from E12.5	Cell death from P0, limited apoptotic signal at P15
Cell proliferation	Reduced number of apical and basal progenitors from E14.5	Reduced number of cortical progenitors from E18.5	Altered balance of apical and basal progenitors	Not mentioned
Neuronal specification/ differentiation	Misspecification of late-born neurons and defect in terminal differentiation of deep-layer neurons	Misspecification of late-born neurons	No major neuronal differentiation defects at early stage of cortical development	Not mentioned
Cell migration and cortical layering	Layering defects: intermixing of deep and upper cortical neurons	Layering defects resulting from defect in late-born neurons generation	Misplacement of newborn neurons at onset of corticogenesis	Normal cortical layering
Neuritogenesis	Not mentioned	Not mentioned	Not mentioned	Reduced dendritic branching and increased apical dendritic spine length in hippocampal neurons at P21

The expression pattern of Cre is reported (gray) on mouse brain slice of various transgenic mice, as indicated. The level of brain slicing correpsonds to the line. (a) De Pietri Tonelli *et al.*[13]; (b) Kawase-Koga *et al.*[16]; (c) Makeyev *et al.*[37]; (d) Nowakowski *et al.*[17]; (e) Davis *et al.*[12]

Dicer in both mouse lines. Indeed, a recent work reported that deletion of Dicer with the Nestin-Cre line was not very efficient as miRNAs were still detected at ~40% of wild-type (WT) levels in E15 Cx.[55]

Early and robust expression of Cre under the regulation of FoxG1 unveiled novel functions for miRNAs at onset of corticogenesis (Table 1). The thickness of the cortical wall of FoxG1:Cre;Dicer:lox/lox embryos was strongly reduced at mid-corticogenesis, presumably resulting from escalating cell death that began after E11.5[37] (Table 1). In this model, interfering with the biogenesis of miRNAs at very early stage in NEPCs did not affect the expression of most neuroepithelial markers (e.g., Sox2, CD133 and musashi1), nor their ability to proliferate (Figure 1). These results suggested that many aspects of NEPC identity were preserved before onset of corticogenesis.[17] Careful histological analyses of E11.5 mutant embryos revealed opposite modification in apical (radial glia cells) and basal progenitor populations size. In addition, the weak expression of ErbB2, Sox9 and Nestin in mutant cortices supported radial glia specification defects. Surprisingly, the population of basal progenitors was scattered through the cortical wall but increased as compared with control through a molecular mechanism that remains unclear.[17] Whether such defects impact on the neuronal output remains debated.[17,37]

Altogether, these results show that mature miRNAs are required from onset of cortical neurogenesis to fine tune expression of genes that control the behavior of distinct populations of cortical progenitors. In addition, the specification of upper-layer neurons seems more dependent on miRNAs than first-born deep-layer neurons. In order to get more insight into the function of miRNAs in cortical progenitors, cortical neural stem cells (NSCs) were derived from either Nestin:Cre;Dicer:lox/lox [55] or Emx1:Cre;Dicer:lox/lox[15] mouse embryos. These cells expressed regular stem cell markers but exhibited peculiar morphologies as compared with control. Dicer-null cortical NSCs retained the ability to self-renew and grow neurospheres, but were highly prone to death when they attempted neurogenesis. Whether these defects reflect the loss of many miRNAs or a selection of few remains to be investigated.

miRNAs orchestrate the behavior of cortical progenitors. In some cases, functional connections to individual miRNAs have been made and at least four specific miRNAs (i.e., let-7b, miR-9, miR-124 and mir-134) were shown to control cell proliferation in the Cx (Figure 1 and Table 2). Let-7b is a member of the let-7 miRNA family, which is expressed in mammalian brain and accumulates during neurogenesis. It regulated cortical progenitor's proliferation and differentiation by targeting two components of the same molecular pathway: TLX, a nuclear receptor that promotes cell cycle progression in the developing brain [56] and the cell cycle regulator cyclin D1.[57] Knockout mice are excellent tools for analyzing gene function *in vivo* but, for technical reason, only few brain miRNAs have been studied with this technology. One example is the miR-9-2/3 double-mutant mouse, which has a significant reduction in the expression of mature miR-9 and its complementary miR-9* and that harbors multiple defects in telencephalic structures. Although the radial glia scaffold was correctly organized, the cerebral Cx of mutant embryos showed enlarged ventricles with hypoplastic cortical upper layers and reduced number of cortical interneurons and Cajal–Retzius (CR) cells. Indeed, miR-9 family members are expressed from the onset of corticogenesis, and regulate directly or indirectly the expression of multiple transcription factors that function in proliferation and differentiation of cortical progenitors, including the direct target genes *FoxG1* and *Meis2* [18] (Table 2). TLX is another target of miR-9, and miR-9 gain of function mediated by *in utero* electroporation decreased ventricular zone (VZ) cell proliferation in developing cerebral Cx.[58] Interestingly, a recent work performed on Xenopus tropicalis suggested that miR-9 also regulates cell survival. In the absence of miR-9, the extra-proliferation of forebrain progenitors was counterbalanced by increased p53-dependent apoptosis, resulting in no cell gain.[59] During corticogenesis, miR-124 accumulates progressively in apical precursors that are undergoing direct

Table 2 Selected miRNAs that contribute to cortical development

miRNAs	Neuronal functions	Target genes	References
Let7-b	Regulates neural stem cell proliferation and differentiation.	*TLX, cyclin D1*	Zhao *et al.*[57]
miR-9	Controls neuronal proliferation and differentiation. Regulates differentiation of Cajal–Retzius cells. Controls axonal pathfinding.	*FoxG1, Meis2, TLX*	Shibata *et al.*,[40] Zhao *et al.*,[58] Shibata *et al.*[18]
miR-34a	Regulates neuronal differentiation. Modulates dendritogenesis, spine morphology, and synaptogenesis.	*SIRT1, Syntaxin-1A, Synaptotagmin-1*	Agostini *et al.*,[72,73] Aranha *et al.*[71]
miR-124	Controls neuronal proliferation and differentiation. Controls neuritogenesis of cortical neurons.	*PTBP1*	Makeyev *et al.*,[37] Maiorano and Mallamaci[38]
miR-125b	Controls dendritic branching. Reduces spine width.	*p250 GAP*	Edbauer *et al.*[77]
miR-128	Controls neural differentiation.	*UPF1*	Bruno *et al.*[70]
miR-132	Controls neurite outgrowth. Regulates dendritic branching and spine density.	*p250 GAP*	Vo *et al.*,[79] Wayman *et al.*,[80] Edbauer *et al.*,[77] Magill *et al.*[81]
miR-134	Regulates cell survival and neuronal migration. Modulates dendritic maturation.	*Chldr-1, Dcx, LimK1, Pumilo-2*	Schratt *et al.*,[39] Fiore *et al.*,[84] Khudayberdiev *et al.*,[85] Gaughwin *et al.*[14]
miR-137	Regulates neural stem cell proliferation and specification. Regulates neuronal migration and dendritogenesis.	*LSD1, Mib1*	Smrt *et al.*,[83] Sun *et al.*[62]

neuronogenesis as well as in basal progenitors. Its overexpression in the Cx stimulated direct neurogenesis and modified the balance between apical and basal progenitors (Table 2). This was the result of an increased transition of neural precursor from apical to basal compartment.[38] Another important regulator of cortical development is miR-134 that promotes neuronal progenitor cells proliferation and counteracts bone morphogenetic protein (BMP) inhibitor chordin-like 1 (Chrdl-1)-induced cell death.[14] Taken together, these data suggest that several miRNAs accumulate in the developing cerebral Cx, some being more critical than others, where they fine-tune expression of multiple genes responsible for the tight regulation of survival, proliferation and specification of cortical progenitors.

The Regulation of Radial Migration by miRNAs Remains Poorly Characterized

The cerebral Cx is a highly organized laminar structure. The first postmitotic neurons migrate outside the neuroepithelium to form the preplate (PP, before E11.5 in mice). This is followed by generation of successive waves of projection neurons that migrate and split the PP into the marginal zone (MZ), mostly containing the CR cells, and the subplate (SP). They generate six layers of neurons in the CP, following an inside-out cell placement (Figure 1, inset). At the onset of corticogenesis, projection neurons reach their final destination by somal translocation, and as the cortical wall thickens, neurons move by locomotion on radial glia fibers.[60,61] Experiments conducted in the cerebral Cx of Emx1:Cre; Dicer:lox/lox embryos suggested that radial migration of projection neurons was impaired and led to lamination defects [13] (Table 1). Moreover, some neurons were misplaced through the depth of the cortical wall in FoxG1:Cre;Dicer:lox/lox mouse embryos. But cell misplacement partly arose as a consequence of loss of radial glia scaffold integrity.[17] Surprisingly, only late-born neurons were misplaced in Nestin:Cre;Dicer:lox/lox embryos, as suggested by BrdU birthdating experiments.[16] Among candidate miRNAs, *in utero* electroporation experiments suggested that miR-137 acts as downstream effector of TLX in cortical progenitors where it controls migration through regulation of a histone lysine-specific demethylase 1 (LSD1).[62] In addition, miR-134 could directly regulate postmitotic cell migration by targeting doublecortin (DCX),[14] a microtubule-associated protein involved in radial migration[63] (Figure 1 and Table 2). However, this miRNA also regulated other targets involved in cell proliferation, suggesting that it orchestrates several developmental steps.[14] Therefore, it is hard to take conclusion with such experiments as miRNAs inhibition takes place in progenitors and may thus indirectly impact on the ability of their progeny to migrate.

It is interesting to note that cortical interneurons were scarcely present in the Cx of miR-9-2/3 double-mutant embryos, suggesting that tangential migration from basal forebrain was impaired after reduction of miR-9 expression.[18]

Forebrain progenitors require a tight regulation of miRNA expression for their proliferation and survival, thus it is premature to conclude on a role of miRNAs as direct regulators of postmitotic neuronal migration. Acute modulation of miRNA expression should be performed in cells that had become postmitotic to decipher whether miRNAs directly regulate neuronal migration in the developing cerebral Cx.

miRNAs Control Differentiation of Cortical Projection Neurons

Impaired differentiation of cortical projection neurons in Dicer conditional knockout embryos. The cellular complexity of cerebral Cx emerges through specification of cortical progenitors into distinct subtypes of neurons that reach deep or upper cortical layers. In addition, the Cx is subdivided into several functional areas where neurons develop selective patterns of gene expression and axonal projections.[64,65] Changes in gene expression underlie transition from progenitors to neurons. Conditional removal of Dicer in the Cx affects this process. Indeed, the cerebral Cx of Emx1:Cre;Dicer:lox/lox embryos has reduced number of Brn1-expressing upper-layer neurons as compared with controls (Figure 1). In addition, the remaining ones were intermingled with Tbr1-expressing deep-layer neurons. Such cell mixing likely resulted from combined defects of cell generation and migration. The post-migratory differentiation of deep-layer neurons was also affected as supported by the strong reduction of FoxP2 expression in layer VI.[66] Likewise, Nestin:Cre;Dicer:lox/lox cortices harbored late-born neuron differentiation defects (E18.5), which were not correlated with poor cell survival after removal of Dicer[16] (Table 1). Consistent with previous observations, the generation of early postmitotic neurons was not affected in FoxG1:Cre;Dicer:lox/lox cortices.[17] Taken together, these observations suggest that differentiation of late-born neurons is more dependent on gene regulation by miRNAs as compared with their deep-layer counterparts. Results obtained *in vitro* with NSC derived from either Nestin:Cre;Dicer:lox/lox or Emx1:-cre;Dicer:lox/lox cortices showed their ability to initiate a differentiation program but failed to terminally differentiate and often harbor abnormal morphologies as compared with their respective control.[15,55] Surprisingly, removal of Dicer from postmitotic projection neurons *in vivo* using Dicer Cre-recombination with a CamKII:Cre mouse line did not lead to major cortical layering defects (Table 1).[12] Thus, it remains unclear to what extent miRNAs regulate all aspects of cortical neuron differentiation, including laminar specification and terminal differentiation *in vivo*.

A network of miRNAs regulates differentiation of cortical neurons. Some miRNAs have been reported as being critical for neural differentiation. These include let-7b, miR-9, miR34a, mir-128 and miR-137 (Table 2 and Figure 1). While let-7b gain of function inhibited NSC proliferation through regulation of Cyclin D1 and TLX, it also accelerated neural differentiation.[57] Likewise, miR-9 regulated cell proliferation and neural differentiation through inhibition of TLX expression.[58] *In situ hybridization* analyses revealed that expression pattern of miR-9 was medio-laterally graded in the Cx, being most intense in the cortical hem where CR cells are born, and it showed a reciprocal gradient to *FoxG1* mRNA expression. Gain- and loss-of-function experiments with

miR-9 modulated the expression of *FoxG1*, which controls generation of CR cells in the developing cerebral Cx *in vivo*.[67–69] Gain-of-function experiments with miR-9 at E11.5 increased differentiation of CR cell whereas its loss resulted in a mild reduction of expression of some CR cell markers *in vivo*.[40] These results suggest that miR-9 regulates differentiation of CR cells through modulation of *FoxG1* expression (Figure 1).

Neural differentiation is partly driven by additional epigenetic mechanisms, some being directly controlled by the brain-enriched miR-128, miR-34a and mir-137 (Table 2). In the developing Cx, miR-128 accumulated in neurons where it controls differentiation by targeting the RNA helicase regulator of nonsense transcripts homolog (UPF1) and the exon-junction complex core component MLN51, thereby repressing nonsense-mediated decay. This pathway degrades both normal and aberrant transcripts harboring stop codons.[70] Another critical miRNA is miR-34a, which orchestrated differentiation of neural progenitors by repressing Sirtuin 1 (Sirt1)[71] and by directly targeting messengers coding for synaptic proteins.[72,73] By affecting LSD1, miR-137 blocked proliferation and promoted migration and differentiation of cortical progenitors. In addition, TLX inhibited transcription of miR-137 by recruiting LSD1 to its genomic region. Thus, miR-137 forms a feedback regulatory loop with TLX and LSD1 to control the dynamics between progenitor proliferation and differentiation during cortical development.[62] Individual miRNAs have specific role during corticogenesis and add novel layers of complexity to the molecular regulation of neuronal differentiation. It is important to note that some miRNAs contribute to the specification and differentiation of glia.[74–76] However, this topic will not be covered here as it is beyond the scope of the present review.

miRNAs Promote Axonal Pathfinding and Dendritogenesis in Development and Plasticity

Disruption of Dicer expression impairs neuron branching and axonal wiring *in vivo*. The directional growth of axon from pyramidal neuron starts while they are migrating in the CP. Many miRNAs are predicted to control expression of molecules important for axonal growth and navigation toward distant cortical and subcortical targets. Indeed, loss of Dicer expression *in vivo* resulted in axonal pathfinding defects. Axons radiated in all directions within the lateral septal nucleus when Dicer was deleted in contrast to the tight fasciculated organization observed in WT mice.[12] Furthermore, miR9-2/3 double-mutant embryos harbored projection malformations, including L1-expressing commissural axons (corpus callosum (CC), anterior commissure (Ac), and fornix/hippocampal commissure (F/Hc)) (Figures 2a and b) as well as cortigofugal axons labeled with TAG-1 and L1.[18]

When projection neurons reach their final position in the cortical wall, they grow dendrites that are progressively decorated with spines at synaptic contacts (Figure 2c). Dendritic spines serve as storage sites for synaptic strength and facilitate transmission of electrical signals to the neuron cell body. Plasticity at synapse is critical for higher cognitive functions and impairment of morphology, and/or quantity of dendritic spines are often associated with aging and central nervous system-related disorders that affect the Cx, including schizophrenia and fragile X mental retardation.[77,78] Interestingly, conditional removal of Dicer in neurons results in reduction of dendritic branching and increased dendritic spine length, which remain, however, functional.[12]

Identification of miRNAs that control neuronal branching and dendrite spine morphology. Several miRNAs have been associated with dendritogenesis, spine regulation and synaptogenesis (Table 2). While we are far from understanding the complex post-transcriptional regulation of these dynamic processes, some studies provide insights into the function of individual miRNAs. The cerebral Cx-enriched miR-34a is one of them, and regulates dendritogenesis and spine morphology during synaptogenesis downstream Tap73, a p53 tumor suppressor gene homolog. Indeed, it has been shown that increasing expression of miR-34a impaired these processes and ultimately led to reduction of inhibitory synapses, possibly resulting from direct targeting of Synaptotagmin-1 and Syntaxin-1 A messengers in terminally differentiating neurons[72,73] (Figure 2c).

When added to cultured neurons, brain-derived neurotrophic factor (BDNF) was shown to increase the expression of miR-132 through the cAMP responsive element-binding protein pathway. This miRNA modulated neurite outgrowth through inhibition of p250GAP, a Rho GTPase-activating protein that regulate spine morphological plasticity.[79,80]

Recent works pointed additional miRNAs, including miR-132 and miR-125b, which are associated with fragile X mental retardation protein in mouse brain and which have opposite functions in dendritic spine regulation and synaptic physiology in hippocampal neurons (Figure 2c and Table 2). Gain-of-function experiments showed that miR-125b induced the formation of longer narrow spines correlated with a reduction of the amplitude of miniature excitatory postsynaptic currents (mEPSC). On the other hand, raising miR-132 level increased both dendritic spine width and mEPSC amplitude. Loss of expression of miR-125b resulted in larger dendritic spines, whereas miR132 downregulation reduced both, dendritic complexity [77] and spine density.[81]

Other critical regulators of dendritogenesis include miR-134 and miR-137. The local dendrite-enriched miR-134[39] accumulated in postmitotic neurons of the developing Cx where it promoted dendrite maturation by sensitizing neural process outgrowth to BMP-4, partly through direct inhibition of the expression of the BMP antagonist Chrdl-1[14,82] (Figure 2). On the other hand, miR-137 was detected in the hippocampus where it controlled dendrite morphogenesis and spine development of young neurons by regulating the translation of mind bomb one (Mib1), an ubiquitin ligase important for neurogenesis.[83]

Dynamic regulation of miRNA's expression contributes to brain plasticity in adulthood. Recent data provide insight into activity-dependent regulation of miRNAs in neurons. Indeed, activity-regulated gene expression contributes to dendritic and synaptic refinements, and recent data further support a role for miRNAs in regulation of these

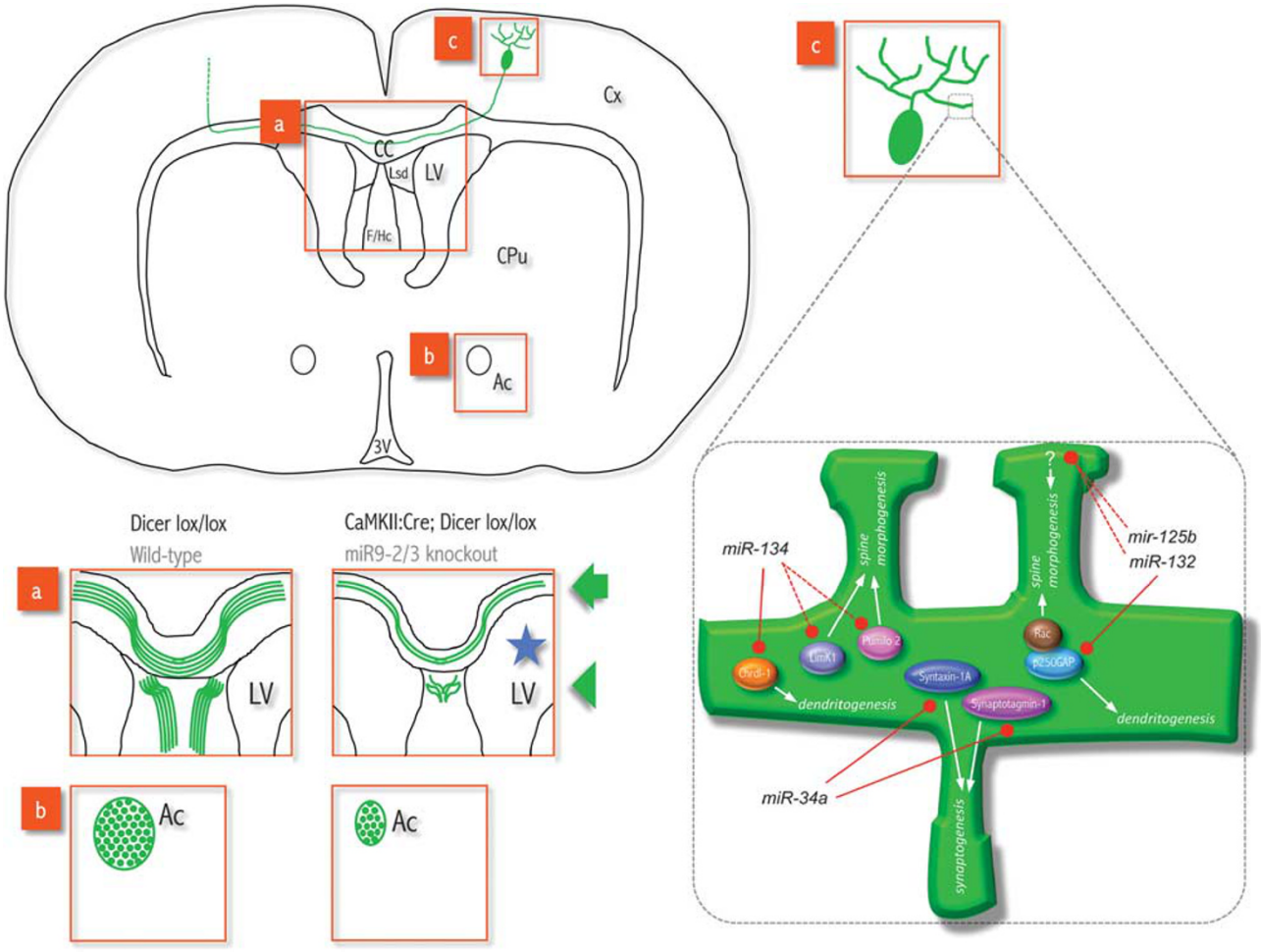


Figure 2 miRNA regulation of axonal pathfinding, dendritogenesis and synaptogenesis. Scheme representing a brain coronal section. Dicer conditional knockout mice (CaMKII:Cre; Dicer:lox/Lox) or miR9-2/3 knockout mice show axonal pathfinding abnormalities (axons are in green). The boxed areas show fasciculation and orientation defects in: (**a**) the CC (arrow), the F/Hc and the Lsd (arrowhead); (**b**) the Ac is reduced in mutants. The blue star shows enlarged ventricle in mutant mice. The boxed area in (**c**) and its zoom-up illustrate a dendrite segment harboring spines. Some miRNAs and their targets regulate dendritogenesis (branching complexity or length, see text), spine morphogenesis (length or width, see text) or synaptogenesis, as indicated on the scheme. Dotted lines refer to target inhibition in hippocampal neurons. LV, lateral ventricle; CPu, caudate-putamen; Lsd, lateral septal dorsal nucleus; 3V, third ventricle

processes to ensure plasticity in the adult brain. Among them, miR-134 is transported in RNA granule to the synapto-dendritic compartment where it controls the expression of key genes, including *LimK1*[39] and *Pumilo 2*,[84,85] thereby regulating dendritic spine size of hippocampal neurons. The miR-134-induced inhibition of *LimK1* expression could be relieved by application of BDNF, a neurotrophin released after elevated neuronal activity.[39] Interestingly, both miR-134 gain and loss of function impaired activity-dependent dendritogenesis, suggesting that miR-134 fine-tuned target expression within a narrow range critical for activity-dependent dendritogenesis. A recent study confirmed the role of miR-134 in synaptic plasticity by demonstrating that overexpression of miR-134 in the CA1 region of the hippocampus abolished long-term potentiation *in vivo* and thus impaired long-term memory formation during contextual-fear conditioning.[86] Activity-dependent dendritic growth required the expression of additional miRNAs, including miR-132 that was originally characterized as a positive regulator of dendritic outgrowth by inhibiting the translation of p250GAP, which control actin cytoskeleton dynamics by promoting Rac activity.[80]

Altogether, these data show that disruption of miRNA processing impairs brain wiring, dendritogenesis and synaptogenesis during development. Moreover, the tight spatio-temporal regulation of miRNAs expression has an important role to support proper dendritogenesis and synaptic plasticity during adulthood.

Concluding Remarks

Accumulating evidence indicates that post-transcriptional mechanisms are essential for the regulation of cortical development. While miRNAs were discovered 20 years ago as part of such mechanism, we just start to understand their contribution to the tight regulation of neurogenesis in the developing cerebral Cx. The finding reviewed here revealed key roles for miRNAs at all developmental stages of corticogenesis as a whole and for generation and differentiation of cortical projection neurons in particular. However, while

miRNAs modulate gene expression at multiple steps of cortical development, their direct contribution to migration of cortical projection neurons remains unclear. In addition, most data have been acquired with Dicer conditional knockout mouse models but functional connections to individual miRNA often remain correlative. The recent development of specific miRNA conditional knockout mice together with the acute modulation of miRNA expression by *in utero* electroporation pave the way to unveil specific function of individual miRNA and to identify their target genes. However, a single miRNA is predicted to target hundreds of different mRNAs,[87] thus much more need to be done to define the full spectrum of genes regulated by a miRNA in a given context. In addition, it will be critical to understand how these multiple targets interact together, and to what extent each of them contribute to assigned miRNA's function in cortical neurogenesis. It will be equally challenging to understand the contribution of miRNAs to neuropathological conditions that affect the Cx and to explore how miRNA-based therapies could normalize aberrant gene regulatory networks in the diseased cerebral Cx.

Conflict of Interest

The authors declare no conflict of interest.

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