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MicroRNA Cluster miR-17-92 Regulates Neural Stem Cell Expansion and Transition to Intermediate Progenitors in the Developing Mouse Neocortex

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SUMMARY

During development of the embryonic neocortex, tightly regulated expansion of neural stem cells (NSCs) and their transition to intermediate progenitors (IPs) are critical for normal cortical formation and function. Molecular mechanisms that regulate NSC expansion and transition remain unclear. Here, we demonstrate that the microRNA (miRNA) miR-17-92 cluster is required for maintaining proper populations of cortical radial glial cells (RGCs) and IPs through repression of Pten and Tbr2 protein. Knockout of *miR-17-92* and its paralogs specifically in the developing neocortex restricts NSC proliferation, suppresses RGC expansion, and promotes transition of RGCs to IPs. Moreover, *Pten* and *Tbr2* protectors specifically block silencing activities of endogenous miR-17-92 and control proper numbers of RGCs and IPs in vivo. Our results demonstrate a critical role for miRNAs in promoting NSC proliferation and modulating the cell-fate decision of generating distinct neural progenitors in the developing neocortex.

INTRODUCTION

In the developing neocortex, self-renewal of neural stem cells (NSCs), proliferation of neural progenitors, and subsequent differentiation are regulated by conserved complex interactions of multiple genes (Guillemot, 2005; Kriegstein et al., 2006; Merkle and Alvarez-Buylla, 2006; Molyneaux et al., 2007; Shen et al., 2006). Radial glial cells (RGCs), the primary cortical neural progenitors, are transformed from neuroepithelial cells/NSCs and reside in the ventricular zone (VZ). Intermediate progenitors (IPs) or basal progenitors are transited from RGCs and populate mostly the subventricular zone (SVZ) (Chenn and McConnell, 1995; Englund et al., 2005; Götz and Huttner, 2005; Haubensak et al., 2004; Noctor et al., 2001; Rakic, 2003). Expansion of NSCs is regulated by both positive and negative factors. For example, ablation of the tumor suppressor gene *Pten* results in a larger cortex and an expansion of NSCs and neural progenitors (Groszer et al., 2001; Zheng et al., 2008), and transcription factor Tbr2 promotes expansion of IPs and elevates IP transition from RGCs (Arnold et al., 2008; Englund et al., 2005; Sessa et al., 2008, 2010). However, the accurate modulation of expression levels of positive and negative regulators that control proper expansion of NSCs and RGCs and transition to IPs is not well understood.

Emerging evidence has shown that microRNAs (miRNAs) play an important role in cortical development. Global depletion of miRNA functions using Dicer ablation results in a smaller cortex and affects NSCs survival and differentiation (Andersson et al., 2010; De Pietri Tonelli et al., 2008; Kawase-Koga et al., 2009, 2010; Nowakowski et al., 2011). miRNAs are frequently transcribed together as polycistronic primary transcripts that are processed into multiple individual mature miRNAs. An important miRNA polycistron is the miR-17-92 cluster and its paralogs miR-106a-363 and miR-106b-25 (Mendell, 2008). Knockout mice of the *miR-17-92* cluster and paralogs display embryonic lethality, indicating a critical role in mouse development (Ventura et al., 2008). miR-17-92 has been shown to be oncogenic and promotes tumorigenesis (Mavrakis et al., 2010; Mu et al., 2009; Olive et al., 2009). Considering the conserved targets of the miR-17-92 family in different tissues, miR-17-92 likely plays a general role in cell proliferation and survival during normal development and under tumorigenesis.

We here show that knockout of the *miR-17-92* cluster restricts expansion of NSCs and RGCs and promotes transition to IPs. miR-17-92 balances the proper RGC and IP populations by suppressing Pten and Tbr2. *Pten* and *Tbr2* mRNA protectors can specifically block endogenous miR-17-92 silencing activities and regulate RGC and IP numbers. Our results demonstrate that the miR-17-92 cluster is an important regulator controlling distinct neural progenitor populations by balancing proper protein output.

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RESULTS

The miR-17-92 Family Promotes Expansion of RGCs and NSCs

The miR-17-92 cluster and paralogs yield 15 mature miRNAs that can be categorized into miR-17, miR-18, miR-19, and miR-92 subfamilies according to their conserved seed sequences (Figure 1A). Due to the function of the miR-17-92 family in regulating cancer cell proliferation, we predicted that miR-17-92 may be essential for neural progenitor expansion in the embryonic cortex. We first examined expression levels of miR-17-92 using the northern blotting assay. All *miR-17-92* subfamilies were expressed in developing mouse cortices, with high expression levels in embryonic day 12.5 (E12.5) cortices and low levels in postnatal day 0 (P0) cortices (Figure S1). Moreover, *miR-17*, *19a*, *92a*, and *106a* displayed strong expression in the VZ in E12.5 cortices detected by in situ hybridization (Figure S1). The high expression of the miR-17-92 family in the VZ suggests their role in cortical NSC and neural progenitor development.

To determine the function of the miR-17-92 family in the developing cortex, we generated mouse models in which *miR-17-92* and its paralogs were genetically ablated by applying the *Cre-loxp* system using *Emx1-Cre* mice due to their specific activity in the cortex (Gorski et al., 2002). *Emx1-Cre* mice were bred with floxed *miR-17-92* transgenic mice to generate *miR-17-92$^{flox/flox}$;Emx1-Cre* mice, called *miR-17-92* single-knockout (*KO*) mice (*miR-17-92 sKO*, *sKO*) (Figure 1B). We also bred *miR-17-92 sKO* mice with *miR-106a-363* or *miR-106b-25* knockout mice, called *XKO* and *5KO*, respectively, to generate double-KO (*dKO*) mice, called *miR-17-92/X-dKO*, *XdKO*, and *miR-17-92/5-dKO*, *5dKO*, respectively (Figure 1B). *miR-17-92 KO* mice displayed slightly reduced cortical thickness at P1, and the reduction was more pronounced at P10 (Figures 1C and S1). Moreover, expression levels of representative miRNAs were decreased in P1 *KO* cortices detected using the northern blotting assay (Figure 1D). miRNAs in *miR-106a-363* and *miR-106b-25* paralogs were not upregulated in *miR-17-92 sKO* cortices as detected by quantitative real-time RT-PCR (qRT-PCR) (data not shown). Our results indicate a successful cortical depletion of miR-17-92 and no significant compensation by the other two paralogs after miR-17-92 deletion.

We next examined miR-17-92 function in neural progenitor and NSC development in *miR-17-92 KO* mice. Neural progenitors can be detected by labeling cells in the S phase with a 30 min pulse of bromodeoxyuridine (BrdU), in the G1, S, G2, and M phase with Ki67, and in the M phase with phospho-histone H3 (PH3). Numbers of BrdU$^+$, Ki67$^+$, and PH3$^+$ cells were not changed in E13.5 *miR-17-92 sKO* and *5dKO* cortices compared to wild-type controls (Figures 1E–1H). However, the numbers and percentages of Pax6$^+$ and Sox2$^+$ RGCs were significantly reduced in E13.5 and E15.5 *miR-17-92 KO* cortices, suggesting a continuous loss of RGCs (Figures 1I–1L; data not shown). There was no significant increase in apoptotic cells in E13.5, E15.5, or P1 *miR-17-92 KO* cortices as detected by a TUNEL assay (data not shown).

We then cultured NSCs collected from E13.5 cortices as neurospheres. After two passages, compared to controls, the numbers and sizes of the neurospheres derived from *miR-17-92 sKO* cortices were reduced (Figure S2). *miR-17*-92-deficient neurospheres properly expressed NSC markers such as Nestin and SSEA-1. However, after a 6 hr pulse of BrdU labeling on attached NSCs, 40% fewer *miR-17*-92-deficient NSCs had incorporated BrdU compared to controls (Figure S2). Our results indicate that, under culture conditions with FGF2 that maintains self-renewal of NSCs and RGCs (Kang et al., 2009), the miR-17-92 cluster enhances growth of cortical NSCs by promoting proliferation.

The miR-17-92 Family Suppresses Transition from RGCs to IPs

We next questioned why the overall number of neural progenitors was not changed while the number of RGCs was reduced in *miR-17-92 KO* cortices. We speculated that the RGC reduction might be caused by abnormal transition to IPs. We divided the Tbr2-expressing area into two subdomains: the "lower Tbr2 domain" (LTD), where IPs are just derived from RGCs in the VZ, and the "upper Tbr2 domain" (UTD), where IPs differentiate into neurons in the SVZ (Munji et al., 2011). We found a significant increase of Tbr2$^+$ IPs in the LTD of E13.5 *miR-17-92 KO* cortices compared with controls, but the number of Tbr2$^+$ cells in the UTD was unchanged or slightly reduced (Figures 2A and 2B). While the number of Tbr2$^+$/BrdU$^+$ was increased, the number of Pax6$^+$/BrdU$^+$ was decreased in *miR-17-92 KO* cortices, indicating that *miR-17-92* depletion promotes transition into proliferative IPs from dividing RGCs (Figures 2C–2F).

We next examined RGC to IP transition by colabeling Tbr2 with Pax6 and Sox2. A significant increase in the numbers of Tbr2$^+$/Pax6$^+$ and Tbr2$^+$/Sox2$^+$ cells was detected in *miR-17-92 KO* cortices, suggesting that more RGCs were in the process of converting into IPs (Figures 2G–2J). The increased transition to IPs from RGCs was maintained in E15.5 *miR-17-92 KO* cortices (Figure S3). However, in E17.5 *miR-17-92 KO* cortices, decreased numbers of Pax6$^+$ RGCs and Tbr2$^+$ IPs were detected, suggesting a reduction of the IP population after E15.5, likely due to progressive loss of RGCs (Figure S4). Furthermore, while *miR-17-92 sKO* cortices showed no changes in the ratio of Tbr2$^+$/BrdU$^+$ cells to Tbr2$^+$ cells throughout development, *5dKO* cortices revealed a transient increase of proliferative Tbr2$^+$ cells at E15.5 followed by a decrease at E17.5 (Figure S4). Our results indicate that the transient increase in total Tbr2$^+$ cells in *miR-17-92 KO* cortices is largely caused by an elevated generation of IPs from RGCs.

Cortical Ablation of the miR-17-92 Family Affects Neuronal Production

To determine whether neuronal production was affected, we examined early-born and late-born neurons. The numbers of Tbr1$^+$ cells were temporarily increased in E13.5 cortices but subsequently decreased in E15.5 and P1 cortices of *miR-17-92 KO* mice (Figures S5A–5C). A transient increase of Satb2$^+$ and Cux1$^+$ neurons was detected in E15.5 cortices, and a decrease of those cells was observed in E17.5 and P1 cortices of *miR-17-92 KO* mice (Figures S5D–S5F; data not shown). We then normalized relative numbers of RGCs and IPs at E13.5, E15.5, and E17.5

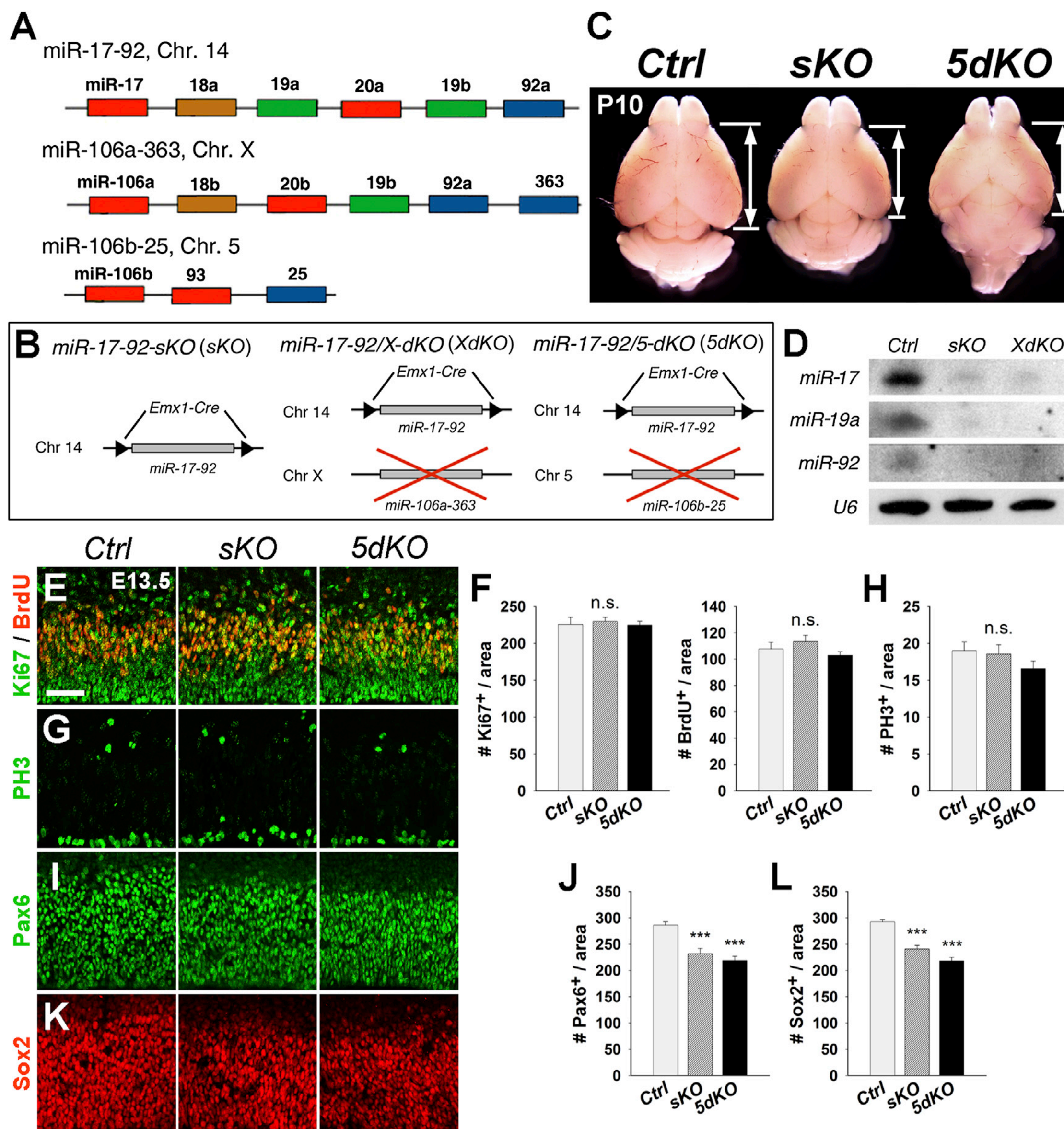

Figure 1. Deletion of the *miR-17-92* Cluster and Its Paralogs Causes Reduced Number of Radial Glial Cells (RGCs) but Not Overall Neural Progenitors in E13.5 Cortices

(A) The schematic genomic organization of the *miR-17-92* cluster and its paralogs miR-106a-363 and miR-106b-25 on the mouse chromosome 14 (Chr. 14), Chr. X, and Chr. 5, respectively.

(B) The strategy of generating *miR-17-92* single- and double-knockout mice using the *Emx1-Cre* line.

(C) Dorsal view of control (*Ctrl*), *miR-17-92 sKO*, and *5dKO* brains at P10. Reduced cortical size was found in *miR-17-92 KO* mice.

(D) Reduced expression levels of representative miRNAs of the *miR-17-92* cluster in *miR-17-92 KO* cortices detected by northern blots. *U6* was used as a loading control.

(E and F) Numbers of $BrdU^+$ and $Ki67^+$ progenitors were unaffected in *miR-17-92 KO* cortices compared to controls.

(G and H) Numbers of $PH3^+$ cells did not show significant changes in *miR-17-92 KO* cortices.

(I–L) Numbers of Pax6- and Sox2-expressing RGCs were decreased in *miR-17-92 KO* cortices.

Scale bar: 50 μm. Data are presented as mean ± SEM; $n \geq 3$ in all genotypes; p values in relation to control (***$p < 0.001$). n.s., not significant. See also Figures S1 and S2.

to those of E13.5 controls. Compared to E13.5 controls, a consistent reduction of RGCs was detected at all three stages in *miR-17-92 KO* cortices, while an increase of IPs was detected at E13.5 and E15.5, and a decrease of IPs was observed at E17.5 (Figure S5G). Moreover, we normalized relative numbers of $Tbr1^+$ and $Satb2^+$ neurons to those of E13.5 and E15.5

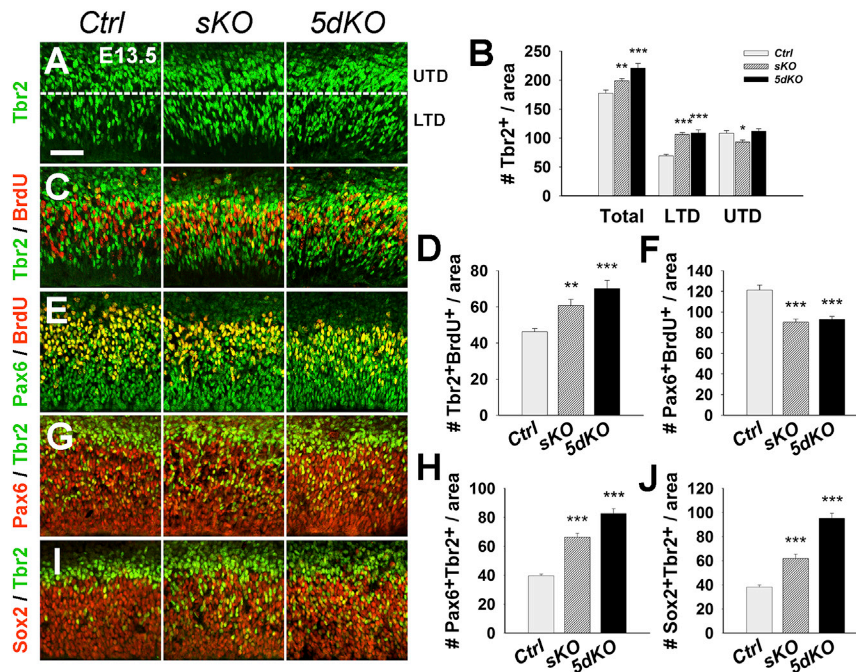

Figure 2. The miR-17-92 Cluster Suppresses Transition of Cortical Intermediate Progenitors

(A) Increased numbers of IPs labeled with Tbr2 in E13.5 *miR-17-92 sKO* and *5dKO* cortices as compared with controls (*Ctrl*). The expression region of Tbr2 was divided into the "lower Tbr2 domain" (LTD) and the "upper Tbr2 domain" (UTD).

(B) Quantification of the total number of IPs and IPs within the UTD and LTD.

(C and D) Numbers of $Tbr2^{+}/BrdU^{+}$ IPs were significantly higher in *miR-17-92 KO* cortices than controls.

(E and F) Numbers of $Pax6^{+}/BrdU^{+}$ RGCs in *miR-17-92 KO* cortices were significantly less compared to controls.

(G–J) Increased transition to IPs from RGCs as detected by $Tbr2^{+}/Pax6^{+}$ and $Tbr2^{+}/Sox2^{+}$ cells in *miR-17-92 KO* cortices compared to controls.

Scale bar: 50 μm. Data are presented as mean ± SEM; n ≥ 3 in all genotypes; p values in relation to control (*$p < 0.03$, **$p < 0.007$, ***$p < 0.001$). See also Figures S3, S4, and S5.

controls, respectively. These neurons were temporarily increased in *miR-17-92 KO* cortices at E13.5 and E15.5, which is consistent with the ectopic transition from RGCs to IPs at these stages (Figure S5H). However, a reduction of both $Tbr1^{+}$ and $Satb2^{+}$ neurons was detected after E15.5 and E17.5, respectively, when the numbers of RGCs and IPs were decreased in *miR-17-92 KO* cortices. Our results suggest that cortical miR-17-92 deletion suppresses RGC expansion and promotes RGC transition to IPs, which results in a transient increase of neuronal production in early developmental stages and a reduction of neurogenesis in late stages, likely due to exhaustion of the RGC pool (Figure S5I).

miR-17-92 Regulates RGC Expansion by Targeting Pten

miRNAs function by silencing target genes. Since the miR-17-92 family promotes RGC expansion and suppresses IP transition, we speculated that their target genes likely negatively regulate RGC expansion and positively direct IP transition. We found that the 3′ untranslated region (3′ UTR) of *Pten* contains two targeting sites for miR-19a (Figure 3A). To validate the targeting effect, we performed a luciferase assay. The 3′ UTR sequence of *Pten* was cloned into a luciferase vector and cotransfected with miR-19a. While luciferase activities in constructs containing the 3′ UTR of *Pten* were not affected by the mutated miR-19a or a control miRNA miR-9, they were significantly reduced by miR-19a (Figure 3B). Furthermore, *Pten* 3′ UTR containing mutations in the seed sequence of miR-19a binding sites abolished miR-19a-targeting effects, proving Pten as a specific target of miR-19a (Figure 3B).

We then tested whether the miR-17-92 silencing effect on Pten is due to degradation of messenger RNA (mRNA) or blockage of translation. Total RNA and protein were extracted from E15.5 control and *miR-17-92 sKO* cortices. *Pten* mRNA levels were not significantly altered between the control and *miR-17-92 sKO* cortices as detected by qRT-PCR (Figure 3C). Conversely, protein levels of Pten were increased in *miR-17-92 sKO* cortices detected by the western blotting assay, indicating a posttranscriptional regulation of Pten by miR-17-92 (Figure 3D).

To validate the specificity of the miR-19 targeting effect on the *Pten* 3′ UTR, we designed *Pten* mRNA protectors, which are complementary sequences that bind to the *Pten* 3′ UTR and block access to the miR-19 targeting sites (Zhang et al., 2012) (Figure S6A). *Pten* protectors showed a blocking effect on miR-19-silencing activities in the *Pten* 3′ UTR in luciferase assays (Figure S6B). We next introduced *Pten* protectors in E13.5 cortices using in utero electroporation, which should partially block endogenous silencing activities of miR-19 and result in phenotypes similar to the upregulation of Pten. The numbers of $Pax6^{+}$ and $Sox2^{+}$ RGCs were significantly decreased, while the number of $Tbr2^{+}$ cells was not greatly changed, after *Pten* protectors were electroporated (Figures 3E–3J). Our results indicate a specific targeting regulation of miR-19 on Pten activity in expanding RGCs (Figure 3K).

miR-17-92 Balances Tbr2 Activity on Transition of RGCs to IPs

Our results have shown that knockout of *miR-17-92* increases generation of IPs in the embryonic neocortex. Because Tbr2 has been shown to promote expansion of IPs, we examined the *Tbr2* 3′ UTR and found a binding site for miR-92a (Figure 4A). We next performed luciferase assays. Luciferase activities in the construct containing the 3′ UTR of *Tbr2* were reduced by miR-92a, but not by the mutated miR-92a or miR-9 (Figure 4B). *Tbr2* 3′ UTR containing mutations in the seed sequence of miR-92a binding sites abolished miR-92a targeting effects,

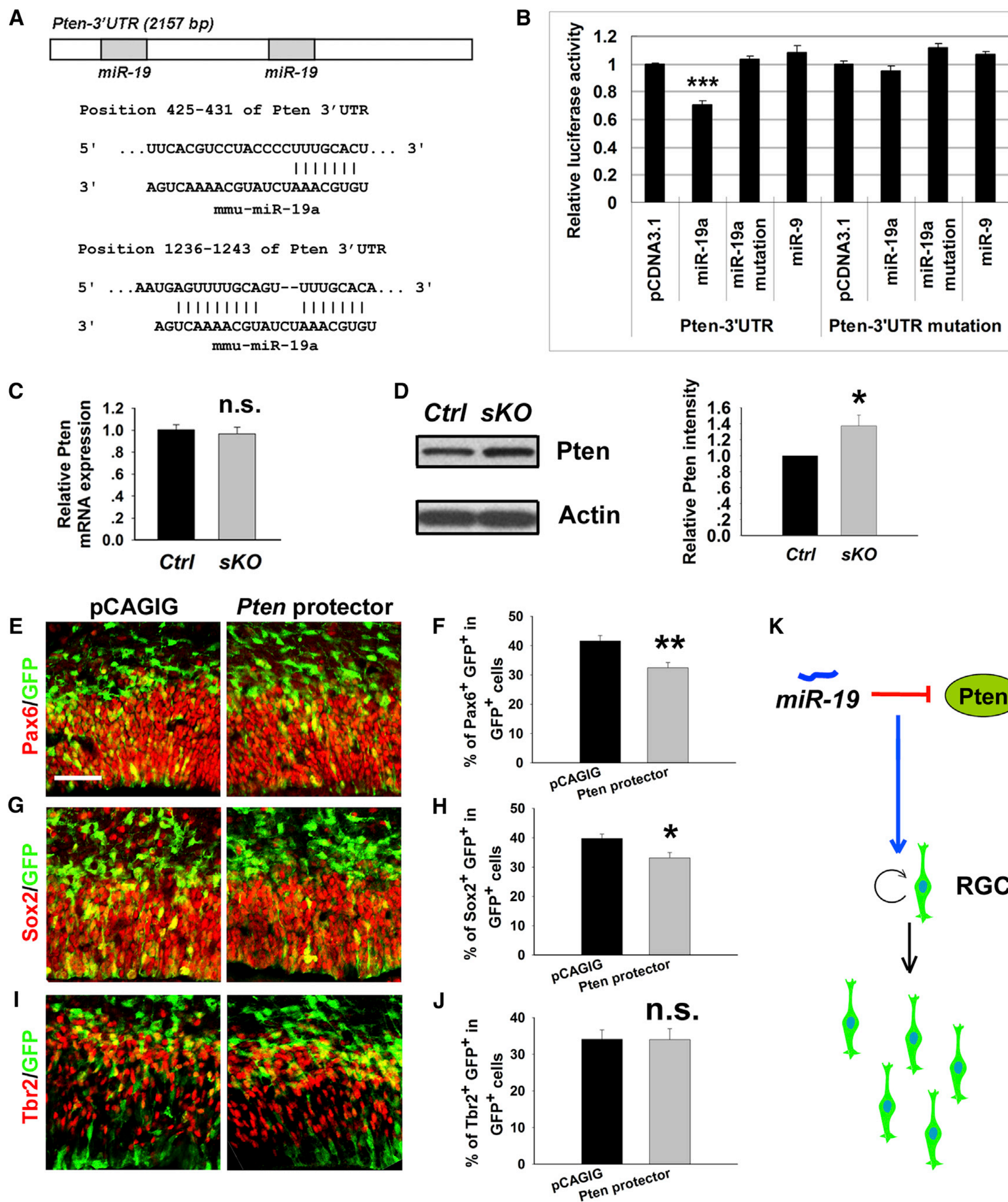

Figure 3. Pten Is a Putative Target Gene of miR-19a in Regulating Cortical RGC Numbers

(A) Predicted targeting sites of miR-19a on the 3′ untranslated region (3′ UTR) of *Pten*.

(B) Luciferase assays of miR-19a targeting effects on the *Pten* 3′ UTR. miR-19a but not the mutation of miR-19a and control miRNA miR-9 recognized the 3′ UTR sequence of *Pten* and reduced luciferase activities. *Pten* 3′ UTR mutation for the seed sequences of miR-19a binding sites abolished miR-19a targeting effects.

(C) *Pten* mRNA level was not affected in E15.5 *miR-17-92 sKO* cortices compared to controls (*Ctrl*), as detected by qRT-PCR.

(D) The protein level of Pten was increased in the E15.5 *miR-17-92 sKO* cortex, detected by western blotting assays.

(E–H) *Pten* mRNA protectors specifically blocked silencing effects of miR-19 in the *Pten* 3′ UTR in vivo. Ectopic expression of *Pten* protectors in E13.5 mouse cortices, analyzed at E14.5, decreased the number of RGCs colabeled with GFP and Pax6 or Sox2.

(I and J) Ectopic expression of *Pten* protectors had no effect on the number of IPs colabeled with GFP and Tbr2.

(K) A summary of miR-19 function on expanding RGCs by repressing Pten.

Scale bar: 50 μm. Data are presented as mean ± SEM; n ≥ 3 in all genotypes; p values in relation to control (*$p < 0.03$, **$p < 0.004$, ***$p < 0.001$). n.s., not significant. See also Figure S6.

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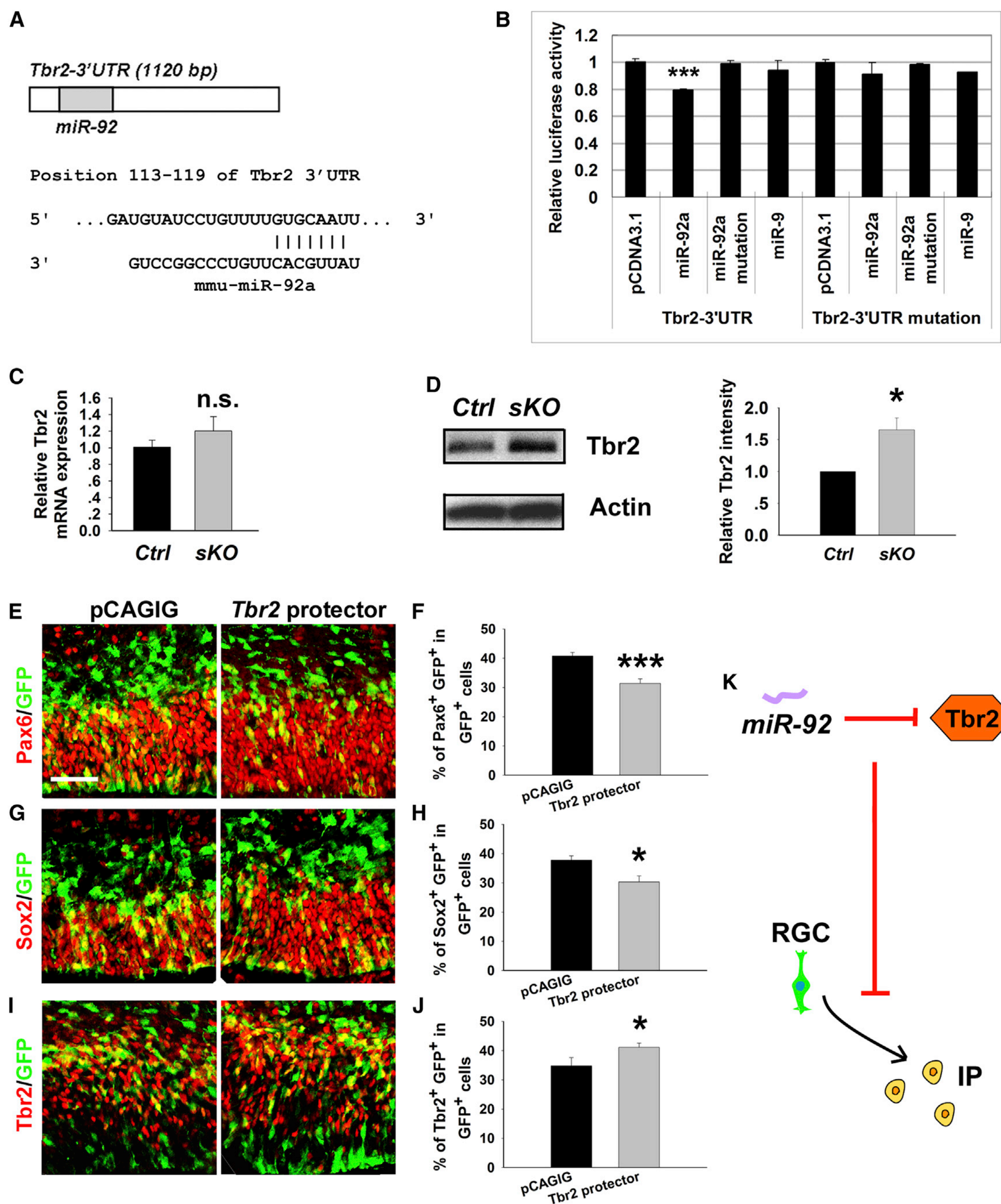

Figure 4. miR-92a Regulates Cortical IP Numbers by Targeting Tbr2

(A) A predicted targeting site of miR-92a on the 3′ UTR of *Tbr2*.

(B) Luciferase activities of the *Tbr2* 3′ UTR construct were greatly reduced by miR-92a, but not by miR-92a mutation and miR-9. *Tbr2* 3′ UTR mutation for the seed sequence of miR-92a abolished miR-92a targeting effects.

(C) *Tbr2* mRNA level was not affected in E15.5 *miR-17-92 sKO* cortices compared to controls (*Ctrl*), as detected by qRT-PCR.

(D) The protein level of Tbr2 was increased in the E15.5 *miR-17-92 sKO* cortex, detected by western blotting assays.

(E–H) Ectopic expression of *Tbr2* protectors in E13.5 mouse cortices, analyzed at E14.5, decreased the number of RGCs colabeled with GFP and Pax6 or Sox2.

(I and J) Ectopic expression of *Tbr2* protectors increased the number of IPs colabeled with GFP and Tbr2.

(K) A summary of miR-92 function on the transition to IPs from RGCs by repressing Tbr2.

Scale bar: 50 μm. Data are presented as mean ± SEM; n ≥ 3 in all genotypes; p values in relation to control (*$p < 0.04$, ***$p < 0.005$). See also Figure S6.

indicating Tbr2 as a specific target of miR-92 (Figure 4B). Moreover, similar to Pten, Tbr2 was regulated at a posttranscriptional level by miR-92 (Figures 4C and 4D).

We next examined the specific targeting effect of miR-92 on Tbr2 expression in neural progenitor development using *Tbr2* mRNA protectors (Figure S6). In E14.5 cortices electroporated with *Tbr2* protectors at E13.5, the numbers of $Pax6^+$ and Sox^+ cells were significantly decreased, while the number of $Tbr2^+$ cells was increased, indicating a specific blockade of silencing activity of the endogenous miR-92 on the *Tbr2* 3′ UTR in regulating IP transition from RGCs (Figures 4E–4K).

DISCUSSION

Maintaining proper populations of distinct neural progenitors is essential for controlling the precise architectural assembly of a functional neocortex. In this study, we have demonstrated an essential role of the miR-17-92 cluster in controlling expansion of NSCs and RGCs, and transition to IPs. Our results reveal an important regulatory mechanism of miR-17-92 in balancing expression levels of target gene Pten, which negatively controls RGC production, and Tbr2, which positively promotes IP generation.

During early neurogenesis, RGCs divide asymmetrically to generate one RGC and one postmitotic neuron (PN) (Molyneaux et al., 2007). As neurogenesis proceeds, RGCs give rise to IPs that normally undergo symmetric division to directly produce PNs (Noctor et al., 2007). Emerging studies have shown that, like coding genes, miRNAs play critical roles in cortical development (Bian and Sun, 2011; Fineberg et al., 2009; Lang and Shi, 2012; Qureshi and Mehler, 2012). We here show that even though the overall numbers of neural progenitors were not changed, a significant reduction of RGCs and an increase of IPs were detected in *miR-17-92 KO* cortices. The elevated transition to IPs from RGCs in *miR-17-92 KO* cortices is likely because more RGCs acquire IP cell fate due to upregulated expression of Tbr2, a putative target of miR-92. Therefore, the normal regulatory function of the miR-17-92 family is to promote the expansion of RGCs and to suppress transition to IPs.

The altered proportions of the RGC and IP populations in the *miR-17-92 KO* cortices ultimately perturb proper neurogenesis. While neuronal production is temporarily increased in *miR-17-92 KO* cortices at E13.5 and E15.5, it is eventually reduced after E17.5 when numbers of both RGCs and IPs are decreased. These phenotypes are likely caused by the following factors. First, the continuous reduction of RGCs may have a more profound impact on neurogenesis than the transient increase of IPs. Second, increased conversion to IPs may progressively exhaust the RGC pool, which eventually results in a reduced IP population. Thus, reduced neurogenesis at perinatal stages is a result of the overall disruption of proportional RGC and IP development when *miR-17-92* is ablated.

Most functional studies of the miR-17-92 cluster have been focused on tumorigenesis, in which miR-17-92 promotes proliferation and survival of tumor cells (Mavrakis et al., 2010; Mu et al., 2009; Olive et al., 2009). In our study, we have validated Pten as a target for miR-19 during cortical development, suggesting a conserved role of miR-17-92 in regulating progenitor expansion. Furthermore, we have identified a target gene of the miR-17-92 cluster—Tbr2, which normally diverts and suppresses RGC development (Arnold et al., 2008; Englund et al., 2005; Sessa et al., 2008). An important role of miR-17-92 is likely to ensure proper protein output of factors such as Pten and Tbr2 in neural progenitors, which, in turn, controls accurate progenitor development and specification, and modulates subsequent neuronal production. Even though the changes of Pten and Tbr2 protein levels by miR-17-92 are relatively moderate, the outcome of progenitor alteration is significant. These results further support the hypothesis that fine-tuning gene regulation by miRNAs plays a critical role in development (Hobert, 2007; Karres et al., 2007).

Because there are four subfamilies with distinct seed sequences within the miR-17-92 family, it is expected that perhaps there are more genes directly affected by the miR-17-92 family in the cortex other than Pten and Tbr2. The next step will be to identify more target genes and establish an interaction network of miR-17-92 and its targets, which collaborate to modulate the expansion and conversion of NSCs and different types of progenitors. Nonetheless, in the current scope of our work, we have demonstrated two major players, Pten and Tbr2, which respond to miR-17-92 silencing activity in cortical NSC and neural progenitor development. Our work has revealed a mechanism of miRNAs controlling distinct progenitor populations in the developing neocortex.

EXPERIMENTAL PROCEDURES

Transgenic Mouse Lines

To knock out the *miR-17-92* cluster in the cortex, *Emx1-Cre* mice (The Jackson Laboratory) were bred with floxed *miR-17-92* mice to generate *miR-17-92$^{flox/flox}$;Emx1-Cre*, called here *miR-17-92* single-knockout (*KO*) mice. *miR-106a-363* and *miR-106b-25* knockout mice were maintained as homozygote (Ventura et al., 2008). To generate double-knockout mice, *miR-17-92$^{flox/flox}$* mice were first bred with *miR-106a-363* or *miR-106b-25* knockout mice, and then bred with *miR-17-92$^{flox/+}$;Emx1-Cre* mice.

For staging of embryos, midday of the day of vaginal-plug formation was considered E0.5; the first 24 hr after birth were defined as P0. Animal use was overseen by the Animal Facility at Weill Cornell Medical College.

mRNA Protectors for *Pten* and *Tbr2*

The mRNA protectors for mouse *Pten* and *Tbr2* were designed as 60 bp complementary sequences covering the miRNA binding sites in their 3′ UTRs. After annealing, they were inserted into the pCAGIG vector for electroporation, and into the pCDNA3.1 vector for the luciferase assay.

Statistics

For the neurosphere-formation assay, three independent experiments were performed. For electroporated mouse sections, at least three brains from each group were analyzed. Statistical comparison was made by an analysis of variance (unpaired Student's t test).

For further details, please refer to Extended Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and Extended Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.03.037.

LICENSING INFORMATION

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