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Role for Lhx2 in corticogenesis through regulation of progenitor differentiation

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ABSTRACT

The neocortex represents the brain region that has undergone a major increase in its relative size during the course of mammalian evolution. The larger cortex results from a corresponding increase in progenitor cell number. The progenitors giving rise to neocortex are located in the ventricular zone of the dorsal telencephalon and highly express Lhx2, a LIM-homeodomain transcription factor. The neocortex fails to form in the Lhx2 constitutive knockout, indicating a role for Lhx2 in corticogenesis, but mid-embryonic lethality of the Lhx2 knockout requires the use of conditional strategies for further studies. Therefore, to explore Lhx2 function in neocortical progenitors, we generated mice with Lhx2 conditionally deleted from cortical progenitors at the onset of neurogenesis. We find that Lhx2 is critical for maintaining the proliferative state of neocortical progenitors during corticogenesis. In the conditional knockouts, the neocortex is formed but is significantly smaller than wild type. We find that deletion of Lhx2 leads to significantly decreased numbers of cortical progenitors and premature neuronal differentiation. A likely mechanism is indicated by our findings that Lhx2 is required for the expression of Hes1 in cortical progenitors, a key effector in the Notch signaling pathway that maintains the proliferative progenitor state. We conclude that Lhx2 regulates the balance between proliferation and differentiation in cortical progenitors and through this mechanism Lhx2 controls cortical size.

Introduction

The neocortex is the largest region of the mammalian cerebral cortex and is responsible for sensory perception, cognition and control of movements. The multilayered and interconnected neurons in the neocortex are formed through a tightly regulated series of cell divisions and migration (Aboitiz et al., 2001; Fish et al., 2008; Nadarajah and Parnavelas, 2002). All neocortical neurons are generated from progenitor cells located in the neuroepithelium lining the lateral ventricle. During development, these neural progenitor cells undergo both symmetric and asymmetric types of division (Noctor et al., 2004, 2008). Cortical neural progenitors change their competency over time, giving rise to distinct types of progenitors or neurons dependent on developmental stage (Alvarez-Buylla et al., 2001; Fishell and Kriegstein, 2003; Gotz and Huttner, 2005; Miller and Gauthier, 2007). Before the onset of neurogenesis the early primary neural progenitor cells, neuroepithelial cells, expand the cortex via symmetric divisions to exponentially generate more progenitors. Around the onset of neurogenesis, the neuroepithelial cells differentiate into radial glia cells (RGCs), which are elongated progenitor cells. RGCs mainly divide asymmetrically to maintain the neural progenitor population while also producing daughter cells that will become either neurons or more restricted progenitors, the basal progenitors (or intermediate progenitors) (Gotz and Huttner, 2005; Kriegstein et al., 2006; Miyata et al., 2010; Noctor et al., 2008).

The precise regulation of cortical progenitor number is critical for determining the number of cortical neurons and cortical size. However, the molecular mechanisms underlying the determination of cortical size are largely unknown. The Notch signaling pathway was shown to play an important role in maintaining cortical neural progenitors in the proliferative state. The deletion of Hes1 and Hes5, two downstream effectors for Notch signaling pathway, leads to premature neurogenesis (Hatakeyama and Kageyama, 2006; Imayoshi et al., 2008; Ishibashi et al., 1995; Kageyama et al., 2008a, 2008b; Ross et al., 2003) and ectopic overexpression of Hes genes prevents neurogenesis (Nakamura et al., 2000; Ohtsuka et al., 1999; Ohtsuka et al., 2001). Thus, Hes proteins sustain progenitors in a proliferative state and inhibit differentiation.

We studied the role for LHX2, a LIM-homeodomain transcription factor, in the regulation of neocortical progenitor proliferation. Within the neocortex, Lhx2 is expressed throughout cortical neurogenesis by neocortical progenitors within the ventricular zone (VZ) of the dorsal telencephalon (dTel). It was previously shown that LHX2 is required for determination of cortical cell fate, as Lhx2 constitutive null mutants display an extremely diminished dTel with an expansion of the cortical hem (Bulchand et al., 2001; Porter et al., 1997). To study the function of Lhx2 in cortical progenitors after the cortex is determined, we generated an Lhx2 floxed line with LoxP sites flanking exons 1 to 3 of Lhx2. We reported previously that when Lhx2 is eliminated in dTel by Emx1–

Abbreviations: cKO, conditional knockout; dTel, dorsal telencephalon; SVZ, subventricular zone; VZ, ventricular zone; WT, wild type.

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Cre at E10.5, the cortical hem does not expand and the neocortex forms (Chou et al., 2009; Mangale et al., 2008). With this conditional deletion at E10.5, the neocortex is disorganized and significantly smaller than WT neocortex, and the fate of progenitors of lateral neocortex is altered and they instead generate an ectopic olfactory cortex (Chou et al., 2009). Although in both the Lhx2 constitutive and Emx1–Cre conditional knockout models the size of the cortex is dramatically reduced, the altered fate of cortical progenitors in both models makes it difficult to determine the role of Lhx2 in regulation of cortical size.

We found when Lhx2 is deleted by Nestin-Cre at E11.5, the fate of cortical progenitors is not changed (Chou et al., 2009). Using this conditional mutant, we demonstrate here that Lhx2 is required for maintaining cortical progenitors in the proliferative state. When Lhx2 is deleted by Nestin-Cre, the neocortex is reduced to less than 50% of the wild type (WT) size. The decreased cortical size is due to dTel neural progenitors exiting the cell cycle and generating neurons prematurely. We find that the deletion of Lhx2 results in a down-regulation of Hes1 expression, which likely contributes to the premature neurogenesis phenotype. Therefore, we have identified a novel molecular mechanism for maintaining cortical progenitors.

Results

Lhx2 controls cortical size

In the Lhx2 constitutive knockout animals, the cortical hem is expanded at the expense of cortical progenitors in the dTel VZ (Bulchand et al., 2001). The lack of neocortical progenitors in the Lhx2 null embryos makes it impossible to study the function of Lhx2 in these progenitors in dTel VZ. We therefore generated mice with an Lhx2 floxed allele (Lhx2 f/f) to enable deletion of Lhx2 after neocortical progenitors in dTel are specified (Chou et al., 2009). In this study, we crossed Lhx2 f/f mice with Nestin-Cre to delete Lhx2 in all neural progenitors (Tronche et al., 1999). In these Lhx2 cKO (Lhx2 f/f; Nestin-Cre), Lhx2 is deleted in dTel from E11.5 (Figs. 1A and A′ and Chou et al., 2009). The cortical hem, marked by the expression of Wnt3a, is not expanded in the Lhx2 cKO (Figs. 1B and B′). At P7, the size of the cortex in the Lhx2 cKO is significantly reduced to less than 50% of the WT size (Figs. 1C, C′ and D, n = 4; $P < 0.001$), and both the length (Fig. 1D′, n = 4; $P < 0.005$) and the width (Fig. 1D″, n = 4; $P < 0.001$) of the cKO cortex are significantly decreased.

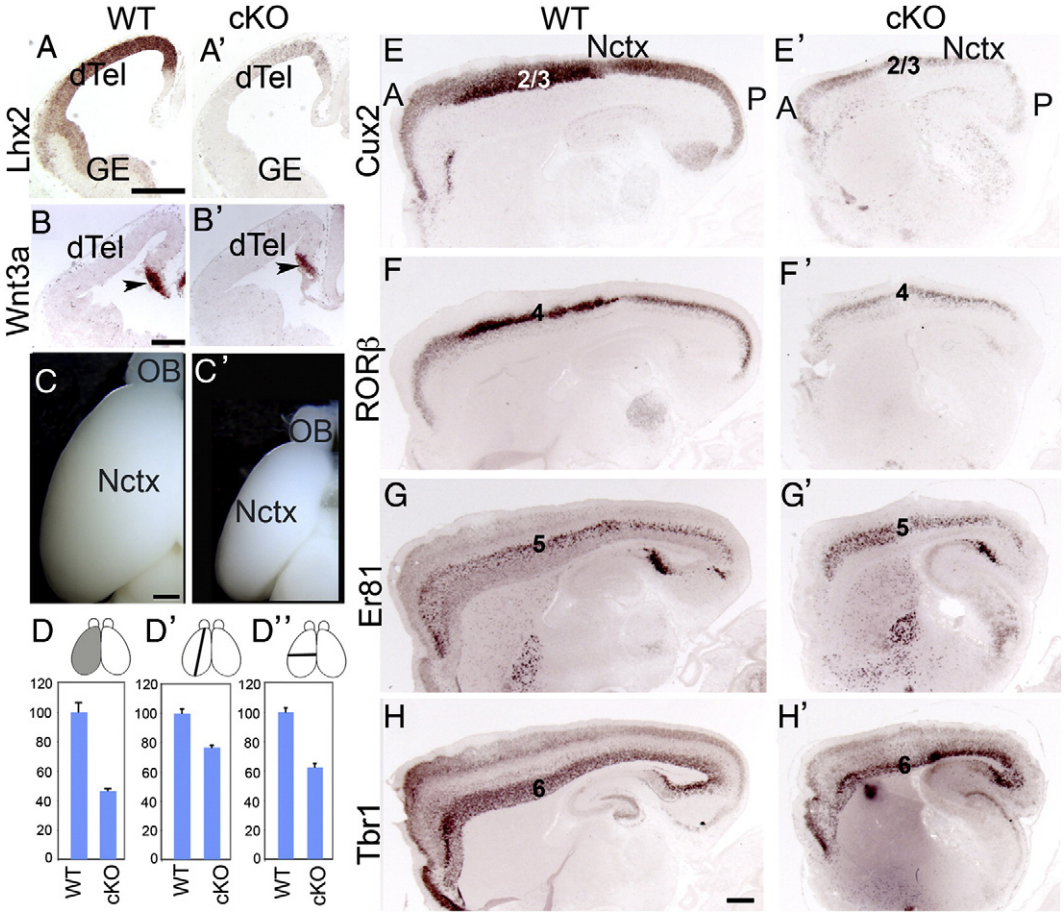

Fig. 1. Conditional deletion of Lhx2 from progenitors at onset of neurogenesis with Nestin-Cre leads to a significantly smaller cortex. (A) In situ hybridization with Lhx2 probe on coronal sections of E11.5 WT (A, Lhx2 f/+) and Lhx2 conditional knockout (cKO) (A′, Lhx2 f/f, Nestin-Cre) brains. Expression of Lhx2 is diminished in the ventricular zone (VZ) of telencephalon in the cKO. (B) In situ hybridization with Wnt3a probe on coronal sections of E13.5 WT and cKO brains. Wnt3a labels cortical hem (arrowheads). The Wnt3a expression domain is similar between WT and cKO. (C) Dorsal view of P7 WT and cKO brains. (D) Histogram of relative surface area of the cerebral cortical hemisphere. The mean of the surface area of the WT cortex is set as 100. Compared with WT (mean ± SEM of 100 ± 6.3, n = 4), the surface area of the cKO cortex (46.4 ± 1.4, n = 4) is significantly decreased ($P < 0.001$ by unpaired Student's *t* test). (D′) Histogram of relative length of the neocortex (Nctx; length from the rostral pole to the occipital pole). The mean of the length of WT neocortex is set as 100. Compared with WT (100 ± 2.9, n = 4), the length of the cKO neocortex (76.6 ± 1.5, n = 4) is significantly decreased ($P < 0.001$). (D″) Histogram of relative width of the neocortex (length from the midline to the lateral side). The mean of the width of WT neocortex is set as 100. Compared with WT (100 ± 2.6, n = 4), the width of the cKO neocortex (62.6 ± 2.2, n = 4) is significantly decreased ($P < 0.001$). (E–H) In situ hybridization with Cux2 (E, E′), RORβ (F, F′), Er81 (G, G′) and Tbr1 (H, H′) probes on sagittal sections of P7 WT and Lhx2 cKO brains. The expression of RORb and Cux2 is greatly reduced in Lhx2 cKO brains. Scale bars: 0.2 mm. A, anterior; dTel, dorsal telencephalon; GE, ganglionic eminence; OB, olfactory bulb; P, posterior.