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Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis

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Abstract

Null mutation of the *Foxg1* gene causes hypoplasia of the mouse telencephalon and loss of ventral telencephalic structures. We show that a crucial early requirement for Foxg1 is in the induction of ventral cell fate in the telencephalon. To study later proliferative defects, we have adapted an iododeoxyuridine and bromodeoxyuridine double labeling protocol for use in the developing embryo, which allows estimation of cell cycle kinetics in a single specimen. This technique is used to demonstrate that the cell cycle is prematurely lengthened in the *Foxg1*-null telencephalon. These defects are first apparent at embryonic day 10.5 (E10.5) and are most severe in the rostral telencephalon. We show that apoptosis is also reduced in the same rostral domain. These defects correspond temporally and spatially with a dramatic reduction in expression of the potent signaling molecule *Fgf 8*. We also show that in the absence of *Foxg1* an excess of neurons is produced from E11.5, depleting the progenitor pool and limiting the growth of the *Foxg1*$^{-/-}$ telencephalon. The increase in neurogenic division coincides with an increase in BMP signaling, as detected by immunohistochemistry for phosphorylated smad-1, -5, and -8. This study reinforces *Foxg1*'s position as a major regulator of telencephalic neurogenesis and supports the idea that *Foxg1* controls precursor proliferation via regulation of Fgf signaling and differentiation via regulation of Bmp signaling.

Keywords: Foxg1; Telencephalon; Cell cycle kinetics; Proliferation; Apoptosis; Differentiation; Bromodeoxyuridine; Iododeoxyuridine; Mouse

Introduction

The embryonic telencephalon arises from the most rostral region of the neural tube, later giving rise to the adult cerebral cortex, hippocampus, olfactory bulbs, and basal ganglia. Soon after its specification, the telencephalon undergoes a period of rapid expansion where the progeny of every cell division re-enters the cell cycle (Bhide, 1996; Caviness et al., 1995). Following this period, there is a gradual slowing of the rate of growth as the progenitor cell cycle lengthens and an increasing proportion of newly born cells exit the cell cycle to differentiate into neurons (Sheth and Bhide, 1997; Takahashi et al., 1996). During this period of telencephalic neurogenesis, the vast majority of neurons that will populate the adult brain for the lifetime of the animal are produced. In order to ensure that the correct types of neurons are produced in the right numbers at the right time and place, the behavior of the telencephalic progenitor cells must be accurately choreographed. Three factors have a major influence on the cellular output from the proliferative zones: the rate of cell proliferation, the rate of cell differentiation, and the rate of cell death. Any failure to correctly regulate any of these crucial processes may have a profound effect on the number of cells produced and the structure of the brain region concerned. Understanding how these processes are modulated at a genetic level remains a major challenge.

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One gene which appears to have an important role in controlling the process of neurogenesis is the winged helix transcription factor *Foxg1* (previously known as BF-1) (Tao and Lai, 1992). Analysis of transgenic mice which lack *Foxg1* provided early evidence that the gene is a key regulator of neurogenesis (Xuan et al., 1995). At embryonic day 9.5 (E9.5), the telencephalon of $Foxg1^{-/-}$ embryos appears grossly similar to that of wild-types. However, from E10.5 until their perinatal death, the telencephalon of *Foxg1*-null mice is noticeably reduced in size. Based on bromodeoxyuridine (BrdU) labeling studies and immunohistochemical staining for markers of differentiated neurons, previous authors have suggested that the causes of the observed telencephalic hypoplasia are 2-fold: reduced progenitor cell proliferation and an increased rate of differentiation (Hanashima et al., 2002; Xuan et al., 1995). These observations point to *Foxg1* being an important regulator of the timing and mode of neurogenesis in the telencephalon.

Here we demonstrate a powerful approach to measuring the cell cycle kinetics of telencephalic neural progenitor cells. Using this technique, we have characterized in detail the proliferation rate of these cells throughout the developing telencephalon in wild-type and $Foxg1^{-/-}$ embryos. As neurogenesis begins (~E10.5), rostrally positioned cells begin to proliferate more slowly in the absence of *Foxg1*. This slowing becomes more widespread and severe as development proceeds, coinciding with an increased rate of telencephalic neurogenesis. We also demonstrate that the level of apoptosis is reduced in the rostral telencephalon of *Foxg1* mutants. Early proliferative and apoptotic defects are shown to coincide with a reduction in the rostral expression of *Fibroblast growth factor 8* (*Fgf8*), thus providing a potential mechanism for the aberrations of the neurogenetic process described here. In addition to characterizing the role of Foxg1 in regulating growth and neurogenesis in the dorsal telencephalon, we show that markers of ventral telencephalic cell fate are never expressed in the $Foxg1^{-/-}$ telencephalon, suggesting that this transcription factor is required for the induction of ventral fates in the telencephalon.

Materials and methods

Animals

$Foxg1^{cre}$ and $Foxg1^{lacZ}$ animals were maintained on a mixed CBA × C57-Bl6 background. In both of these alleles, all but the first 13 amino acids of the *Foxg1* coding sequence is replaced by *cre recombinase* (Hebert and McConnell, 2000) or *lacZ* reporter (Xuan et al., 1995) coding sequences. *Foxg1*-null embryos were obtained from timed matings of *Foxg1* heterozygous mice. $Foxg1^{cre/cre}$, $Foxg1^{lacz/lacz}$, and $Foxg1^{cre/lacz}$ embryos were all observed to have the same phenotypic abnormalities, in agreement with previous authors (Hebert and McConnell, 2000; Pratt et al., 2002), and are denoted $Foxg1^{-/-}$. In all analyses, *Foxg1* heterozygous embryos were found to be phenotypically identical to wild-type as previously described (Pratt et al., 2002; Xuan et al., 1995) and in some cases were included in the control group. The day of the vaginal plug following mating was designated E0.5. Embryonic and adult tissues were genotyped by PCR as described before (Pratt et al., 2004).

Injection of S-phase tracers

For double labeling experiments, pregnant females were injected intra-peritoneally with 200 μl of 100 μg/ml (in 0.9% NaCl) iododeoxyuridine (IddU) (Sigma) and then 1.5 h later with the same dose of bromodeoxyuridine (BrdU) (Sigma) and sacrificed after 30 min. For 8 h BrdU cumulative labeling, four BrdU injections were given at 0, 2, 4, and 6 h before sacrifice at 8 h.

Calculation of cell cycle kinetic parameters

In the telencephalic neuroepithelium, precursors progress through the cell cycle asynchronously (Takahashi et al., 1993). In such a population of proliferating cells, the fraction of cells in a given phase of the cell cycle is directly proportional to the length of that phase relative to the total length of the cell cycle (Nowakowski et al., 1989). BrdU and IddU are halogenated thymidine analogues that are incorporated into DNA synthesized during S-phase. Sequentially exposing proliferating cells to IddU and BrdU allows us to differentiate between defined populations of cells. The relative sizes of these populations allow us to calculate the total cell cycle time (T_c) and the length of S-phase (T_s) of the proliferating pool. According to this technique, telencephalic progenitors are exposed to IddU in vivo at $T = 0$ h such that all cells in S-phase at the beginning of the experiment are labeled with IddU (Fig. 1A). At $T = 1.5$ h, cells are exposed to BrdU to label all cells in S-phase at the end of the experiment (S_{cells}) (Fig. 1A). These cells will also be labeled with IddU, which is still present in the bloodstream. Animals are killed at $T = 2$ h. It takes approximately 30 min for injected IddU and BrdU to circulate and label the DNA of S-phase cells to detectable levels (Nowakowski et al., 1989); therefore, the interval during which cells can incorporate IddU but not BrdU (T_i) is 1.5 h (Fig. 1A). Since telencephalic neural precursors are not synchronized, cells in the initial IddU-labeled S-phase cohort will leave S-phase at a constant rate during T_i. Consequently, this leaving fraction (L_{cells}) will be labeled with IddU but not BrdU, as summarized in Fig. 1A. By staining tissue sections with monoclonal antibodies that allow us to distinguish cells labeled with just IddU from those which incorporated BrdU and IddU, we can count the L_{cells} and S_{cells} fractions (see Figs. 1B and C). The ratio of the length of any one period of the cell cycle to that of another period is equal to the ratio of the number of cells in the first period to the number in the second period (Nowakowski et al., 1989). Therefore, the ratio