Neuron
Report

Fezf2 Expression Identifies a Multipotent Progenitor for Neocortical Projection Neurons, Astrocytes, and Oligodendrocytes

Chao Guo,[1,3] Matthew J. Eckler,[1,3] William L. McKenna,[1] Gabriel L. McKinsey,[2] John L.R. Rubenstein,[2] and Bin Chen[1,*]
[1]Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA 95064, USA
[2]Department of Psychiatry, Neuroscience Program, and the Nina Ireland Laboratory of Developmental Neurobiology, University of California, San Francisco, CA 94158, USA
[3]These authors contributed equally to this work
*Correspondence: bchen@ucsc.edu
http://dx.doi.org/10.1016/j.neuron.2013.09.037

SUMMARY

Progenitor cells in the cerebral cortex sequentially generate distinct classes of projection neurons. Recent work suggests the cortex may contain intrinsically fate-restricted progenitors marked by expression of Cux2. However, the heterogeneity of the neocortical ventricular zone as well as the contribution of lineage-restricted progenitors to the overall cortical neurogenic program remains unclear. Here, we utilize in vivo genetic fate mapping to demonstrate that Fezf2-expressing radial glial cells (RGCs) exist throughout cortical development and sequentially generate all major projection neuron subtypes and glia. Moreover, we show that the vast majority of CUX2$^+$ cells in the VZ and SVZ are migrating interneurons derived from the subcortical telencephalon. Examination of the embryonic cortical progenitor population demonstrates that Cux2$^+$ RGCs generate both deep- and upper-layer projection neurons. These results identify Fezf2$^+$ radial glial cells as a multipotent neocortical progenitor and suggest that the existence, and molecular identity, of laminar-fate-restricted RGCs awaits further investigation.

INTRODUCTION

The neocortex contains six layers of projection neurons and glia. Projection neurons in each cortical layer display similar morphologies, axonal projections, and gene expression patterns (Kwan et al., 2012). During development, cortical projection neurons are generated from radial glial cells (RGCs) and basal progenitors in an inside-out pattern such that deep-layer neurons are generated first, followed by upper-layer neurons (Molyneaux et al., 2007). Three decades of work based upon transplantation experiments (Desai and McConnell, 2000; McConnell, 1985; McConnell and Kaznowski, 1991), viral lineage tracing (Luskin et al., 1988; Walsh and Cepko, 1988), and in vitro culture of single RGCs (Shen et al., 2006) suggests that cortical projection neuron subtype is sequentially determined by birthdate through progressive lineage restriction of a common RGC (Leone et al., 2008). However, the identification of early Cux2-expressing (Cux2$^+$) RGCs, which were reported to be intrinsically specified to generate late-born, upper-layer neurons (Franco et al., 2012), calls into question this decades-old model and raises the possibility that deep-layer projection neurons are similarly generated from lineage-restricted progenitors (Franco and Müller, 2013; Marín, 2012).

The transcription factor *Fezf2* (also known as *Fezl* and *Zfp312*) is expressed in early cortical progenitors and deep-layer neurons and is critical for the fate specification of subcerebral projection neurons (Chen et al., 2005a, 2005b; Molyneaux et al., 2005). In *Fezf2*$^{-/-}$ mice, subcerebral projections are absent and deep-layer neurons instead switch their identity to become corticothalamic or callosal projection neurons (Chen et al., 2005a, 2005b, 2008; Han et al., 2011; McKenna et al., 2011; Molyneaux et al., 2005). Several studies suggest that ectopic expression of *Fezf2* in late cortical progenitors (Chen et al., 2008) or immature neurons (De la Rossa et al., 2013; Rouaux and Arlotta, 2013) redirects these cells to differentiate into subcerebral projection neurons. These results indicate that expression of *Fezf2* in cortical progenitors may be sufficient to confer a subcerebral neuron identity, and thus Fezf2-expressing (Fezf2$^+$) cortical progenitor cells may be lineage restricted to generate deep-layer neurons (Franco and Müller, 2013; Woodworth et al., 2012).

To investigate the lineage potential of Fezf2$^+$ progenitor cells, we performed in vivo genetic fate mapping using the *Fezf2* locus. Here we show that Fezf2$^+$ cortical progenitor cells are RGCs that exist throughout cortical neurogenesis. Temporal fate mapping demonstrated that Fezf2$^+$ RGCs sequentially generate projection neuron subtypes and glia based upon the birthdate of these cells. Furthermore, Fezf2$^+$ RGCs generated upper-layer neurons without expressing detectable levels of CUX2 protein. Finally, we demonstrate that cells labeled by *Cux2-Cre* and *Cux2-CreER^{T2}* generate both deep- and upper-layer projection neurons. Collectively, these results indicate that Fezf2$^+$ RGCs are a multipotent progenitor for neocortical projection neurons, astrocytes, and

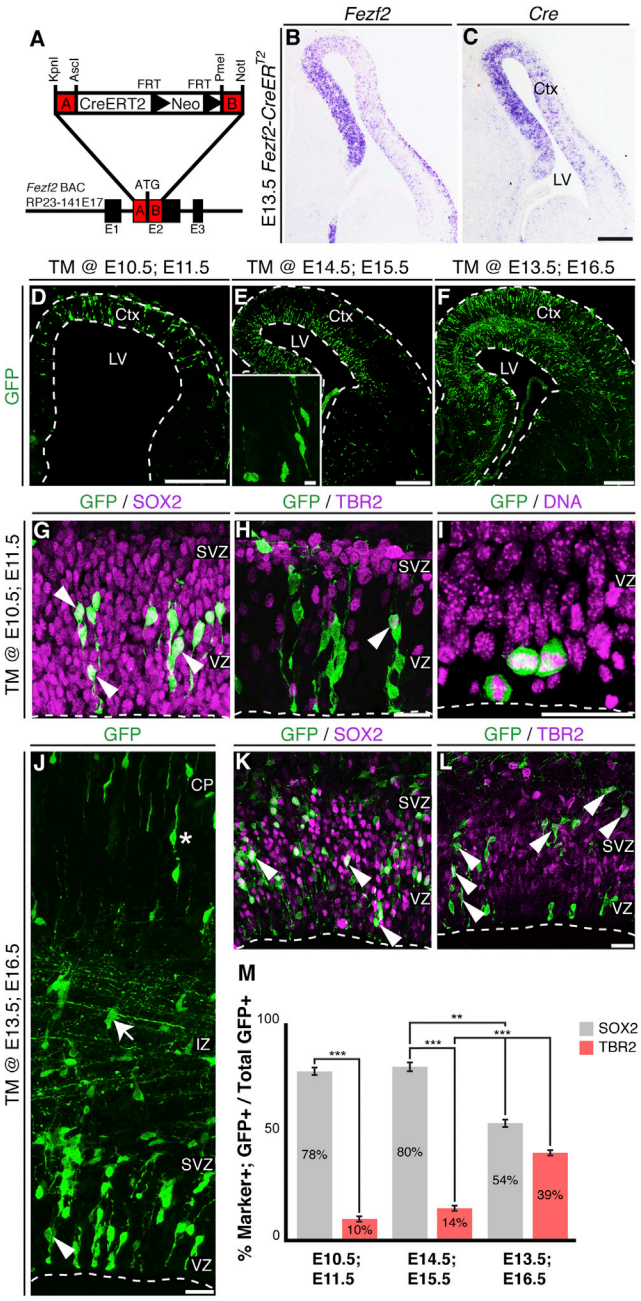

Figure 1. *Fezf2*-Expressing Progenitors Are RGCs

(A) Strategy for generation of *Fezf2-CreER*^{T2} mice.

(B and C) In situ hybridization for *Fezf2* (B) and *Cre* (C) at E13.5.

(D–F) Low-magnification images of GFP$^+$ cells in the cortex of *Fezf2-CreER*^{T2}*; RCE-GFP* mice following CRE-mediated recombination.

(G and H) Twenty-four hours after tamoxifen induction, most GFP$^+$ cells expressed SOX2 (78% ± 3%) (G), and few cells expressed TBR2 (10% ± 2%) (H).

(I) GFP$^+$ cells dividing at the ventricular surface.

(J–L) In TM at E13.5; E16.5 brains, Fezf2$^+$ RGCs gave rise to basal progenitors. GFP was expressed in both ventricular zone (VZ) and subventricular zone (SVZ) progenitors (arrowhead), migrating neurons in the intermediate zone (arrow), and cortical neurons (asterisk) (J). GFP$^+$ cells expressed SOX2 (54% ± 3%) and showed typical RGC morphology (K). Many GFP$^+$ cells expressed TBR2 (39% ± 2%) (L).

(M) Quantification of the percentages of GFP$^+$SOX2$^+$ RGCs and GFP$^+$TBR2$^+$ basal progenitors among all the GFP$^+$ cells ± SEM. **p < 0.005, ***p < 0.0001; CP, cortical plate; Ctx, cerebral cortex; IZ, intermediate zone; LV, lateral ventricle; TM, tamoxifen; SVZ, subventricular zone; VZ, ventricular zone.

Scale bars: 250 μm (C–F), 10 μm (E, inset), and 25 μm (H–J and L). See also Figure S1.

oligodendroctytes and suggest that laminar-fate-restricted RGCs remain to be identified.

RESULTS

Lineage-Traced Fezf2-Expressing Progenitor Cells Are RGCs

We first characterized *Fezf2* expression by in situ hybridization. As previously reported (Hirata et al., 2004), we detected *Fezf2* expression in early neocortical progenitors (Figure 1B). Interestingly, *Fezf2* expression in the ventricular zone (VZ) persisted postnatally, long after deep-layer neuron generation has ceased (Figure S1A available online). This was confirmed by GFP expression in *Fezf2-GFP* bacterial artificial chromosome (BAC) transgenic mice (Gong et al., 2003; Shim et al., 2012), which revealed GFP$^+$ cells in the VZ during late embryonic and early postnatal stages (Figure S1B). To assess the differentiation potential of Fezf2$^+$ progenitor cells, we generated nine independent *Fezf2-CreER*^{T2} BAC transgenic mouse lines (Figure 1A). In situ hybridization for *Cre* and *Fezf2* showed that *Cre* expression was identical to that of endogenous *Fezf2* (Figures 1B and 1C; Figures S1C and S1D). Breeding these mice to three different *Cre* reporter lines (*RCE-GFP*, *R26R-LacZ*, or *TauR-mGFP*; Friedrich and Soriano, 1991; Hippenmeyer et al., 2005; Sousa et al., 2009) revealed that the fused CreER^{T2} protein was tightly regulated by tamoxifen (Figures S1E–S1F′). Although *Cre* mRNA was expressed in deep-layer neurons (Figures 1C and S1D), we observed tamoxifen-induced recombination in these neurons with only the *TauR-mGFP* reporter (Figures S1H–S1I). No recombination was observed in postmitotic neurons upon tamoxifen administration with the *Rosa26R-LacZ* or *RCE-GFP* reporters (Figures S1G, S1J, and S1J′). Critically, this allowed us to perform lineage-tracing experiments for Fezf2$^+$ cortical progenitor cells using the *RCE-GFP* reporter without the ambiguity caused by Cre-mediated recombination in postmitotic neurons.

Examination of *Fezf2-CreER*^{T2}*; RCE-GFP* mice after tamoxifen induction revealed that recombination specifically marked Fezf2$^+$ RGCs (Figures 1D–1M). Twenty-four hours after tamoxifen administration, ~80% of GFP$^+$ cells expressed the RGC marker SOX2, whereas ~10% of GFP$^+$ cells expressed the basal progenitor marker TBR2 (Figures 1D, 1E, 1G, 1H, and 1M). The majority of GFP$^+$ cells were located in the VZ; many had both apical and basal processes and divided at the ventricular surface (Figures 1G–1I), all of which are characteristic of RGCs. The few TBR2$^+$GFP$^+$ cells were likely basal progenitors newly generated from Fezf2$^+$ RGCs. Supporting this, 3 days after an embryonic day 13.5 (E13.5) tamoxifen administration (TM at E13.5; E16.5), the fraction of TBR2$^+$GFP$^+$ cells increased to 39% (Figures 1F and 1J–1M). These results indicate that lineage-traced Fezf2$^+$ progenitors are RGCs.