Original Article

Spindle Orientation-Independent Control of Cell Fate Determination by RGS3 and KIF20A

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Abstract

It was proposed that similar to its role in the invertebrate nervous system, mitotic spindle orientation (or cell cleavage plane orientation) of a dividing neural progenitor cell specifies the fate of daughter cells in the mammalian brain, modulating the production of neurons via symmetric versus asymmetric cell divisions during the course of neurogenesis. Experimental tests of the sufficiency of spindle/cleavage plane orientation in mammalian cell fate determination have yielded conflicting results. On the other hand, the necessity of spindle/cleavage plane orientation in mammalian cell fate determination has not yet been addressed. Here we examined the necessity of spindle/cleavage plane orientation in mammalian cell fate determination in mice with loss-of-function of the RGS3-KIF20A interaction axis. We present evidence that while inactivation of RGS3 or KIF20A was linked to a shift in neural progenitor cells from proliferative to differentiative divisions in the developing cortex, these genetic mutations did not lead to anticipated alteration in the orientation of spindle/cleavage plane. Our results indicate that the RGS3-KIF20A axis regulates the balance between proliferation and differentiation in the mammalian cortex employing a mechanism independent of spindle/cleavage plane orientation. These data also caution against using spindle/cleavage plane orientation as the synonym for cell fate determination.

Key words: cell cleavage plane, cell fate determination, cerebral cortex, KIF20A, mitotic spindle orientation, neural progenitor cells, proliferation versus differentiation, RGS3

Introduction

Radial glial cells (RGCs), the primary neural progenitor cells (NPCs) of the mammalian cerebral cortex, are highly polarized cells with the nucleus located in the ventricular zone (VZ) and a long radial process connecting to the pial surface. During development, RGCs first proliferate to expand their population and later progressively enter asymmetric cell divisions to sequentially generate neurons and glia. Given the similar polarized nature of invertebrate NPCs and the importance of mitotic spindle orientation, or more precisely cell cleavage plane orientation, in asymmetric cell division in the invertebrate nervous system (Homem and Knoblich 2012; Homem et al. 2015; Venkei and Yamashita 2018), a causal link between spindle/cleavage plane orientation of mitotic RGCs and daughter cell phenotype in the mammalian brains was suggested, based on the observation of correlations between spindle/cleavage plane orientation and asymmetric inheritance during cortical neurogenesis (Chenn and McConnell 1995). The mitotic spindle orientation...
model hypothesized that horizontal divisions (cleavage planes perpendicular to the ventricular surface) are preferentially symmetric progenitor self-renewals, while vertical divisions (cleavage planes parallel to the ventricular surface) tend to be neurogenic. It was later revised that neurogenic divisions include both oblique and vertical divisions (Kosodo et al. 2008), as strictly vertical divisions are rare in the mammalian cortices. The mitotic spindle orientation model predicted that as cortical development progresses from the early phase of progenitor expansion to the phase of active neurogenesis, spindle/cleavage plane orientations correspondingly shift from preferentially horizontal divisions to more oblique and vertical divisions, causing cleavage furrow bypassing the apical membrane domains that may harbor key cell fate determinants. However, analyses of spindle/cleavage plane orientations in dividing RGCs at neurogenic stages of cortical development obtained data inconsistent with the hypothesis. For examples, analyses of angles of divisions in the developing mouse and rat cortices have found that RGCs at the ventricle mainly take the form of horizontal divisions (Stricker et al. 2006; Konno et al. 2008; Noctor et al. 2008), an observation that is difficult to reconcile with massive neuron production during the period of cortical neurogenesis. Time-lapse imaging of cortical slice cultures revealed complex rotation and oscillation of the mitotic spindle before progenitor cell division; however, the data also found that cleavage orientation was not a valid predictor of cell phenotype as disparate daughters can be generated by the same spindle behavior and orientation (Haydar et al. 2003). Time-lapse imaging studies also indicated that the majority of asymmetric RGC divisions segregate the apical membranes to both daughter cells (Matsuzaki and Shitamukai 2015; Delaunay et al. 2017), an observation inconsistent with segregation of the apical cell fate determinants hypothesized by mitotic spindle orientation model. In addition, experimental exploration of the mitotic spindle orientation model by modulating the activity of some key polarity-related factors identified in the invertebrate studies have so far yielded conflicting results in the mouse cortex. For instances, G_{iβγ} complex, but not G_{i} subunit, of the heterotrimeric G proteins was reported to regulate cell fate in cortical NPCs based on mitotic spindle orientation analyses (Sanada and Tsai 2005); however, our genetic studies of mice carrying a constitutively active G_{i} subunit revealed that G_{i} subunits are strong initiators of neuronal fate in daughter cells (Murai et al. 2010). Modulation of the activity of LCN/GSPM2, a modulator of G protein signaling, caused randomization of mitotic spindle orientation (Konno et al. 2008) and affected the rate of outer radial glia generation (Shitamukai et al. 2011), a phenomenon also observed in the study of human NPCs (LaMonica et al. 2013), but did not induce a switch from symmetric self-renewal divisions to asymmetric neurogenic divisions (Konno et al. 2008). Other experiments of perturbing microtubule- or spindle-regulating factors have also reported divergent results with respect to a possible role of spindle/cleavage plane orientation in cell fate determination (Fish et al. 2006; Yingling et al. 2008; Gauthier-Fisher et al. 2009; Postiglione et al. 2011; Mora-Bermudez et al. 2014). Most importantly, however, while all studies to date have investigated whether spindle/cleavage plane orientation may influence the mode of cell division during mammalian neurogenesis, the flip side of the question, i.e., whether the orientation of spindle/cleavage plane is required for controlling proliferative versus neurogenic cell divisions in cell fate specification remains untested.

We have found that the EphrinB1-RGS3-KIF20A axis of protein–protein interaction network and the G_{i} subunit work together to regulate the balance between proliferation and differentiation in the developing mouse cortex. Genetic inactivation of Ephrin-B1 (Qi et al. 2008), PDZ-RGS3 (RGS3 isoform 1) (Qi et al. 2010), mitotic kinesin KIF20A (Geng et al. 2018), or genetic activation of G_{i} subunit (knockin of an RGS-insensitive G_{i2} mutant, G184S/G_{i2}) (Murai et al. 2010) causes cortical NPC to leave the cell cycle, leading to a shift of the balance in RGCs from proliferation to neuronal differentiation. We reasoned that these strains of mutant mice would present a suitable genetic platform to test the role of spindle/cleavage plane orientation in cell fate determination. Should spindle/cleavage plane orientation be functionally essential for cell fate choice, i.e., symmetric proliferative division versus asymmetric neurogenic division, these mice would reveal changes in orientations in favor of neuronal differentiation. Specifically, these mice are expected to show a switch in progenitor cells from horizontal divisions (progenitor expansion) to vertical or oblique divisions (neuronal differentiation). In this report, we present our analyses of the in vivo patterns of cell cleavage planes in dividing RGCs of the Pdz-Rgs3 and Kif20a knockout mice. Our data found no detectable connection between the cell fate-regulating function of RGS3 or KIF20A and cleavage plane orientation.

Materials and Methods

Embryonic Brain Sample Preparation

Animal procedures were approved by the Institutional Animal Care and Use Committee. Preparation of brain samples from the littermate wild-type and mutant mice was previously described (Qi et al. 2010; Geng et al. 2018). Briefly, wild-type and mutant embryonic brains were dissected out at E11.5 or E15.5, fixed in 4% paraformaldehyde at 4°C overnight, cryoprotected in 30% sucrose, and embedded in OCT. Brain sections of 12 μm thickness were obtained, stained with Hoechst 33258 (1 μg/ml), and mounted with fluoromount G. Sections used in this study were from the same brain samples used in phenotype analyses in the previous two studies (Qi et al. 2010; Geng et al. 2018). Brain images were taken using a confocal microscope (Zeiss LSM 510 Upright 2 photon) at ×40 optical view.

Cell Cleavage Plane Analysis

Apically localized dividing progenitor cells at Anaphase/Telophase were identified with the appearance of typically condensed separating chromatids. Angles of cleavage planes with reference to the surface of the VZ were determined using Image-Pro Premier (Media Cybernetics, Inc). The data of each individual brain were obtained from 30 to 50 pictures of brain sections at ×40 optical view.

Statistical Analysis

Kolmogorov–Smirnov (K–S) test was used to compare distribution of cell cleavage plane angles between mutant and wild-type brains from the Rgs3 and Kif20a knockout mice. No statistically significant differences between mutant and wild-type divisions in the three data groups could be detected.

Results

We began by examining the planes of division in cortical NPCs of the Pdz-Rgs3 germline knockout mice at embryonic day 11.5 (E11.5), an early stage of progenitor cell expansion. As the cortex contains various types of progenitor cells, including RGCs, outer
subventricular zone progenitor cells (Fietz et al. 2010; Hansen et al. 2010; Wang et al. 2011), intermediate progenitor cells (IPC) or basal progenitor cells (Haubensak et al. 2004; Miyata et al. 2004; Noctor et al. 2004), and short neural progenitor cells (Tyler and Haydar 2013), we focused our analyses on dividing RGCs located at the apical surface. To investigate the in vivo patterns of cell cleavage plane distribution, we stained sections of E11.5 brains with nuclear dye and documented confocal images. To maintain consistency in analyses, we focused on dividing progenitor cells with the following two characteristics: 1) being localized at the apical surface of the VZ (for scoring apical RGCs) and 2) showing condensed separating chromatids (for conspicuously identifying dividing cells at Anaphase/Telophase of mitosis). The angles of the cleavage planes were determined with reference to the apical surface of the VZ (Fig. 1A). Consistent with what was observed in previous studies (Stricker et al. 2006; Konno et al. 2008; Noctor et al. 2008), most of the occurring cell divisions were horizontal divisions in the cortices of the wild-type brains (Fig. 1B). Our data showed that distributions of cleavage plane orientations among littermate brains of E11.5 were also largely comparable with no obvious deviation from the predominant feature of the occurring horizontal cell divisions (Table 1 and Fig. 1B).

To further evaluate cleavage plane orientation in relation to cell fate determination, we next analyzed cleavage planes in mice with germline knockout of the Kif20a gene. Our previous analyses of homozygous mutant brains and their wild-type littermates showed that knockout of Kif20a resulted in early cell cycle exit and precocious neuronal differentiation of cortical NPCs (Geng et al. 2018). Examination of cleavage plane orientations between these mutant and wild-type littermate brains showed that the predominant pattern of horizontal divisions observed during normal cortical neurogenesis was also preserved in the homozygous Kif20a mutant brains (Table 2 and Fig. 2B). Together, these results showed that there were no discernible differences in cell cleavage plane orientations of cortical NPCs in these two strains of knockout mice, although RGS3 and KIF20A and vertical divisions at this developmental stage. We next examined the mutant brains of the Pdz-Rgs3 knockout mice at E15.5 (Fig. 2A), a peak stage of cortical neurogenesis when we previously observed an early cell cycle exit and precocious neuronal differentiation in these mutant brains (Qiu et al. 2010). Our data showed that distributions of cleavage plane orientations among littermate brains of E15.5 were also largely comparable with no obvious deviation from the predominant feature of the occurring horizontal cell divisions (Table 2 and Fig. 2B).
Table 1. Analysis of cleavage planes of apical mitotic cells in the E11.5 cortices of the Pdz-Rgs3 germline knockout mice. (A) Summarizes the analysis of cleavage plane distribution in 15° intervals in each individual littermate brains (three wild-type and three homozygous mutant brains). The data of each brain represent the analyses of anaphase/telophase cells from 30–50 confocal pictures of ×40 optical view (examples are shown in Figure 1A). (B) is the combined data from (A) showing the cleave plane distributions in wild-type or mutant brains.

| Angles | Genotypes | +/+ | −/− |
|--------|------------|-----|-----|
| 0–15   |            | 1   | 1   |
| 15–30  |            | 2   | 1   |
| 30–45  |            | 6   | 4   |
| 45–60  |            | 9   | 5   |
| 60–75  |            | 11  | 6   |
| 75–90  |            | 34  | 18  |

Table 2. Analysis of cleavage planes of apical mitotic cells in the E15.5 cortices of the Pdz-Rgs3 germline knockout mice. (A) Summarizes the analysis of cleavage plane distribution in 15° intervals in individual littermate brains (three wild-type and three homozygous mutant brains). (B) is the combined data from (A) showing the cleave plane distributions in wild-type or mutant brains.

| Angles | Genotypes |
|--------|------------|
| 0–15   | 4   | 3   |
| 15–30  | 9   | 6   |
| 30–45  | 11  | 10  |
| 45–60  | 19  | 19  |
| 60–75  | 41  | 35  |
| 75–90  | 102 | 91  |

Discussion
Initially formulated to explain neuronal differentiation process in the Drosophila neuroblast system, the mitotic spindle orientation hypothesis presented an intriguing model for understanding how symmetric versus asymmetric cell divisions might be balanced during development (Homem and Knoblich 2012; Homem et al. 2015; Venkei and Yamashita 2018). During the past two decades, the notion of a causal link between spindle/cleavage plane orientation and cell fate determination has been extended into studies of the mammalian brain and was thought to be a major mechanism governing the control of proliferation versus differentiation during mammalian neurogenesis (Homem et al. 2015). More recently, the same concept was also introduced to...
Figure 2. Analysis of cell cleavage plane orientation in E15.5 cortex of the Pdz-Rgs3 and Kif20a germline knockout mice. (A) Examples of apical divisions with various angles in the E15.5 cortices. Scale bar represents 100 μm. (B) The graphs show distributions of cleavage plane angles at 15° intervals in brains of the wild-type and Pdz-Rgs3 knockout mice (percentages were based on Table 2B). (C) The graphs show cleavage plane angle distributions in brains of the wild-type and Kif20a knockout mice (percentages were based on Table 3B). K-S test showed that there were no significant differences in distributions of cleavage plane angles between mutant Rgs3 or Kif20a and wild-type brains.

several other stem/progenitor cell systems with epithelial or polarized characteristics (e.g., Kulukian and Fuchs 2013; Williams and Fuchs 2013). Furthermore, spindle/cleavage plane orientation defects were proposed to contribute to brain malformation and some neurological disorders, such as microcephaly and lissencephaly, or tumorigenesis, as a result of postulated imbalance of proliferation versus differentiation during stem/progenitor cell development. However, to date, conclusive evidence demonstrating the sufficiency of spindle/cleavage plane orientation for cell fate determination concerning the control of proliferation versus differentiation in the mammalian brain is still lacking (Peyre and Morin 2012; Shitamukai and Matsuzaki 2012; Matsuzaki and Shitamukai 2015; Delaunay et al. 2017) and a direct causal role of spindle/cleavage plane orientation in the
Table 3. Analysis of cleavage planes of apical mitotic cells in the E15.5 cortices of the Kif20a knockout mice. (A) Summarizes the analysis of cleavage plane distribution in 15° intervals in individual littermate brains (four wild-type and four homozygous mutant brains). (B) is the combined data from (A) showing the cleave plane distributions in wild-type or mutant brains.

| Angles | Genotypes |
|--------|-----------|
|        | +/+       | −/−       |
| 0–15   | 4 #1 3 #2 | 6 #1 2 #2 |
| 15–30  | 9 7 #1 | 9 6 5 #2 |
| 30–45  | 12 7 5 #1 | 15 10 | 12 2 3 |
| 45–60  | 18 7 5 #1 | 33 11 5 | 11 1 3 |
| 60–75  | 34 7 5 #1 | 45 25 5 | 39 4 3 |
| 75–90  | 123 7 5 #1 | 112 54 5 | 111 19 18 |

Notes

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Spindle Orientation Not Required for Cell Fate Determination  Qiu et al.  | 7

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