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Ferret–mouse differences in interkinetic nuclear migration and cellular densification in the neocortical ventricular zone

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ABSTRACT

The thick outer subventricular zone (OSVZ) is characteristic of the development of human neocortex. How this region originates from the ventricular zone (VZ) is largely unknown. Recently, we showed that over-proliferation-induced acute nuclear densification and thickening of the VZ in neocortical walls of mice, which lack an OSVZ, causes reactive delamination of undifferentiated progenitors and invasion by these cells of basal areas outside the VZ. In this study, we sought to determine how VZ cells behave in non-rodent animals that have an OSVZ. A comparison of mid-embryonic mice and ferrets revealed: (1) the VZ is thicker and more pseudostratified in ferrets. (2) The soma and nuclei of VZ cells were horizontally and apicobasally denser in ferrets. (3) Individual endfeet were also denser on the apical (ventricular) surface in ferrets. (4) In ferrets, apicalward nucleokinesis was less directional, whereas basalward nucleokinesis was more directional; consequently, the nuclear density in the periventricular space (within 16 μm of the apical surface) was smaller in ferrets than in mice, despite the nuclear densification seen basally in ferrets. These results suggest that species-specific differences in nucleokinesis strategies may have evolved in close association with the magnitudes and patterns of nuclear stratification in the VZ.

1. Introduction

The outer subventricular zone (OSVZ), which contains undifferentiated progenitor cells (OSVZ [or basal] radial glia-like cells, oRG [bRG] cells), is a characteristic developmental feature of the human neocortex (Zecevic et al., 2005; Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). The OSVZ is also evident in primates (Smart et al., 2002; Kelava et al., 2012; Betizeau et al., 2013) and ferrets (Fietz et al., 2010; Reillo et al., 2011; Martinez-Cerdeno et al., 2012; Reillo and Borrell, 2012). Although rodent neocortical primordia do not have cytoarchitechtonically distinct OSVZ-like structures, they do have oRG-like progenitors (though much less abundantly than in primates and ferrets) in regions basal to the ventricular zone (VZ), i.e., in the subventricular zone (SVZ) or intermediate zone (Shitamukai et al., 2011; Wang et al., 2011; Martinez-Cerdeno et al., 2012; Tabata et al., 2012). How oRG cells emerge, and how the OSVZ is maintained and expanded, are important questions in the context of an evolutionary and pathological understanding of the human neocortex (LaMonica et al., 2012; Lewitus et al., 2013). It is clear that oRG cells originate from the VZ, but how they leave the VZ and accumulate basally to form the OSVZ is largely unknown.

We recently obtained experimental evidence that acute alterations of density and/or traffic of progenitor cell nuclei within the VZ can lead to abnormal separation of a certain fraction of undifferentiated progenitors from the VZ (Okamoto et al., 2013). When we induced over-proliferation of undifferentiated (Pax6$^+$) progenitors via artificial expression of Wnt3a in early embryonic mouse neocortical walls, nuclear packing density in the VZ increased, concomitant with a slight thickening of the VZ. This experimentally induced nuclear densification was followed by abnormal exit of Pax6$^+$ progenitors from the VZ and invasion by these cells into the basal neuronal territory. Similar Pax6$^+$ heterotopia also occurred when we imposed another acute physical load on VZ cells by inhibiting their interkinetic nuclear migration (INM): progenitors that were overcrowded periventricularly as a result of insufficient basalward nucleokinesis (due to removal of their basal processes, induced by knockdown of the cell-surface molecule TAG-1) left the VZ and invaded the neuronal territory. Based on further examinations carried out using physical techniques (such as laser ablation), as well as in silico mechanical simulations, we proposed that progenitors evacuate (or are forced to exit) from the VZ in response to excessive acute mechanical stress (Okamoto et al., 2013).

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Those results, obtained in mice, and previous suggestions on the expansion of progenitors' proliferative zones under the influence of spatial limitations (Smart, 1965; Charvet and Striedter, 2011; Poluch and Juliano, 2013) together prompted us to wonder whether the evolutionary emergence of OSVZ progenitors might have been influenced by mechanical factors within the VZ. Vertical mitotic spindle orientation (and resultant cytokinesis perpendicular to the apical surface) can contribute to the supply of non-apically-connected VZ cells that move basally and eventually adopt an oRG-like morphology (Konno et al., 2008; Postiglione et al., 2011; Shitamukai et al., 2011; LaMonica et al., 2013); therefore, regulation of the cleavage orientation of stem-like cells at the apical surface may underlie the evolutionary changes that have generated OSVZ. Another factor to consider from an evolutionary standpoint is nuclear traffic within the VZ. The neocortical VZ is much thicker in human than in mouse (Zecevic, 1993; Bayer and Altman, 2006). Because the thickness of the VZ is defined by the extent of pseudostratification along the apicobasal axis, i.e., by how many nuclei exhibiting INM are staggered from the apical surface toward the basal side (Boulder Committee, 1970; Takahashi et al., 1993; Miyata, 2008; Taverna and Huttner, 2010), it is possible that as more nuclei are stratified within the VZ (i.e., as the VZ becomes thicker), nuclear traffic in the VZ becomes heavier. If so, different mammalian species may have evolved different strategies for cellular management of VZ nuclear traffic. Therefore, it is necessary to perform comparative studies involving quantitative analysis of cellular behavior in the VZ.

In this study, we compared the embryonic neocortical VZ of the ferret (a gyrencephalic mammal) and the mouse (which is lissencephalic). Although cytogenesis in the ferret neocortical primordium has previously been studied by a variety of approaches used widely in rodents (McSherry, 1984; Chenn and McConnell, 1995; Reid et al., 1997; Chenn et al., 1998; Martinez-Cerdeno et al., 2006; Fietz et al., 2010; Martinez-Cerdeno et al., 2012; Reillo and Borrell, 2012; Poluch and Juliano, 2013), the absolute thickness of the VZ and other parameters that might potentially relate to the physical condition of VZ cells have not been compared between ferrets and rodents. Our findings were as follows: (1) the VZ is thicker (i.e., stratifies more nuclei) in ferrets than in mice. (2) The soma of each VZ cell was more slender (i.e., the major axis/minor axis ratio was greater) in ferrets. (3) Horizontal nuclear packing density in the basal part of the VZ was greater in ferrets, whereas the density in the periventricular area ($\leq$16 μm from the apical surface) was smaller in ferrets. (4) The average area of individual endfeet comprising the apical surface was smaller in ferrets. Furthermore, we performed mean-squared displacement (MSD) analysis (Norden et al., 2009; Leung et al., 2011; Okamoto et al., 2013) on nuclear movements, and found that (5) both apicalward and basalward INM phases in ferret VZ are quite different from those observed in mice.

2. Materials and methods

2.1. Animals

Pregnant ferrets were obtained from Marshall BioResources (Tsukuba, Japan). Pregnant ICR mice were obtained from SLC (Hamamatsu, Japan). All protocols for animal experiments were approved by The Animal Care and Use Committee of the Nagoya University.

2.2. Immunofluorescence

Cross-sectional and tangential immunohistochemistry were performed as described previously (Okamoto et al., 2013). Brains were fixed with periodate–lysine–paraformaldehyde (PLP) fixative (McLean and Nakane, 1974), immersed in 20% sucrose, embedded in OCT compound (Miles), frozen, and sectioned coronally and tangentially (16 μm). Frozen sections were treated with the following primary antibodies: anti-Pax6 (rabbit, PRB-278P, COVANCE), anti-ZO-1 (mouse, 33-9100, Invitrogen), or anti-GFP (rat, 04404-84, Nacalai Tesque; chick, GFP-1020, Aves Labs). After washes, sections were treated with secondary antibodies conjugated to Alexa Fluor 488, Alexa Fluor 546, or Alexa Fluor 647 (Molecular Probes, A-11029, A-11006, A-11034, A-11030, A-11035, A-11081, A-21236, A-21245) and subjected to confocal microscopy (Olympus FV1000).

2.3. Plasmids

For live observation, sporadic visualization was achieved by electroporation with a mixture of conditional expression plasmids, EFX-LPL-LynN-EGFP (0.5 μg/μl) and BA-LPL-H2B-mRFP1 (0.5 μg/μl), and a Cre-recombinase expression plasmid, EFX-cre (0.001 μg/μl), as described previously (Okamoto et al., 2013).

2.4. Exo utero electroporation

Pregnant ferrets were anesthetized with isoflurane and subjected to exo utero surgery (Muneoka et al., 1986) so that exteriorized embryos (E28–29) could be clearly recognized. Electroporation was performed as previously described (Saito and Nakatsuji, 2001; Kawasaki et al., 2012), with some modifications. DNA solution containing Fast Green was injected bilaterally into the lateral ventricle. The head of each ferret embryo was placed between electrodes (3-mm diameter, forceps type; CUY650P3, NEPAGENE), and 55-V electric pulses were applied four times.

2.5. In utero electroporation

In utero electroporation was performed using pregnant ICR mice, as described previously (Okamoto et al., 2013). Forceps-type disk electrodes (3-mm diameter) were used, and 33-V electric pulses were applied four times.

2.6. Live observation of neural progenitor cells

Cross-sectional and en face cultures of cerebral walls were prepared from ferret and ICR mouse embryos as described previously (Miyata et al., 2004; Okamoto et al., 2013). Ferret and mouse cerebral walls, labeled by electroporation and treatment with the lipophilic styryl dye FM4-64 (Kawaue et al., 2014), were microsurgically processed and mounted in glass-bottomed dishes using collagen gel. Time-lapse confocal microscopy was performed using an inverted CV1000 system (Yokogawa) with a 40× objective lens (UPLFLN 40XD, numerical aperture (NA) 0.75, Olympus) or an upright CSU-X1 microscope (Yokogawa) with a water-immersion 20× objective lens (XLUMPlanFLN 20XW, NA 1.00, Olympus) equipped with an iXon + CCD camera (Andor). Chambers for on-stage culture were filled with 40% O_2.

2.7. Quantitative analysis of nuclear migration

Mean-square displacement was obtained from time-dependent changes of nuclear position along the apicobasal axis, as described previously (Okamoto et al., 2013). To the mean-squared-displacement profiles, we fit the power-law model $\mathrm{MSD}\ (\Delta t) = 2D\,\Delta t^{\alpha}$, where t is time, D is diffusivity (mobility), and α is directionality. D and α were estimated by linear regression of ln(MSD) versus ln(t) (using KaleidaGraph): $\ln(\mathrm{MSD}(\Delta t)) = \ln(2D) + \alpha \ln(\Delta t)$, and the intercept and slope