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Dynamics of the cell division orientation of granule cell precursors during cerebellar development

Satoshi Miyashita [a,b,1], Toma Adachi [a,c,1], Mariko Yamashita [a,d], Takayuki Sota [b], Mikio Hoshino [a,*]

[a] Department of Biochemistry and Cellular Biology, National Institute of Neuroscience, NCNP, Tokyo 187-8502, Japan
[b] Department of Electric Engineering and Bioscience, Graduate School of Advanced Science and Engineering, Waseda University, Tokyo 169-8555, Japan
[c] Department of Life Science and Medical Bioscience, Graduate School of Advanced Science and Engineering, TWIns, Waseda University, Tokyo 162-8480, Japan
[d] Department of Development and Regenerative biology, Graduate School of Medical and Dental Science, Tokyo Medical and Dental University, Tokyo 113-8510, Japan

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ABSTRACT

The cerebellar granule cell (GC) system provides a good model for studying neuronal development. In the external granule layer (EGL), granule cell precursors (GCPs) rapidly and continuously divide to produce numerous GCs as well as GCPs. In some brain regions, the orientation of cell division affects daughter cell fate, thus the direction of GCP division is related to whether it produces a GCP or a GC. Therefore, we tried to characterize the orientation of GCP division from embryonic to postnatal stages and to identify an environmental cue that regulates the orientation. By visualizing chromatin in EGL GCPs at M-phase, we found that the directions of cell divisions were not random but dynamically regulated during development. While horizontal and vertical divisions were equivalently observed in embryos, horizontal division was more frequently observed at early postnatal stages. Vertical division became dominant at late cerebellar developmental stages. Administration of a SHH inhibitor to cultured cerebellar slices resulted in randomized orientation of cell division, suggesting that SHH signaling regulates the direction of cell division. These results provide fundamental data towards understanding the development of GCs.

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1. Introduction

During development, the balance and the timing of proliferation and differentiation must be strictly controlled in certain tissues. Dysfunction often results in diseases or disorders, including tissue dysplasia or tumors (Ernst, 2016; Gonda and Ramsay, 2015). Numerous studies have been carried out to understand proliferation and/or differentiation of cells, but the underlying molecular machinery remains elusive.

Cerebellar granule cell precursors (GCPs) provide a fascinating model to study this issue, because (1) developmental processes and gene expression profiles are well characterized, (2) development of GCPs continues even after birth, (3) availability of easy gene transfer techniques to GCPs, and (4) established slice culture experiments for the developing cerebellum. GCPs originate from the embryonic rhombic lip, migrate anteriorly along the pial surface and begin to form the external granule cell layer (EGL) (Machold and Fishell, 2005; Wang et al., 2005; Hatten and Roussel, 2011). In the outer EGL, GCPs rapidly proliferate to produce daughter cells (Fujita et al., 1966). Some of the daughter cells exit the cell cycle and migrate into the inner EGL, where they become GCs. These post-mitotic GCs migrate through the molecular layer to reach the internal granule cell layer where they are integrated into the neural circuit (Komuro and Yacubova, 2003). Although these processes are well described, it is still elusive how the proliferation and differentiation of GCPs and GCs are regulated.

The orientation of the cleavage plane of dividing cells can be one of the key elements to regulate daughter cell fate (Zhong and Chia, 2008). In the ferret cerebral cortex, time-lapse imaging studies have revealed that vertical division (cleavage plane is parallel to the apico-basal axis) of a radial glial cell (RGs) tends to produce two daughter RGs, while horizontal division is prone to generate one neuron and one RG (Chenn and McConnell, 1995). These findings indicate that, in the cerebellum, the proper regulation of cleavage planes may be required to control daughter cell fates, which eventually contributes to coordination of proper proliferation and differentiation of GCPs and GCs.

Here, we analyzed the orientation of cleavage planes of dividing GCPs in both sagittal and coronal section from E16.5 to P15. We found that the orientation was not random but precisely regulated. Additionally, the tendency of the orientation dynamically changed as development proceeded. Furthermore, administration of a SHH inhibitor to

* Corresponding author at: Department of Biochemistry and Cellular Biology, National Institute of Neuroscience, NCNP, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8502, Japan.
E-mail address: hoshino@ncnp.go.jp (M. Hoshino).
[1] These authors contributed equally.

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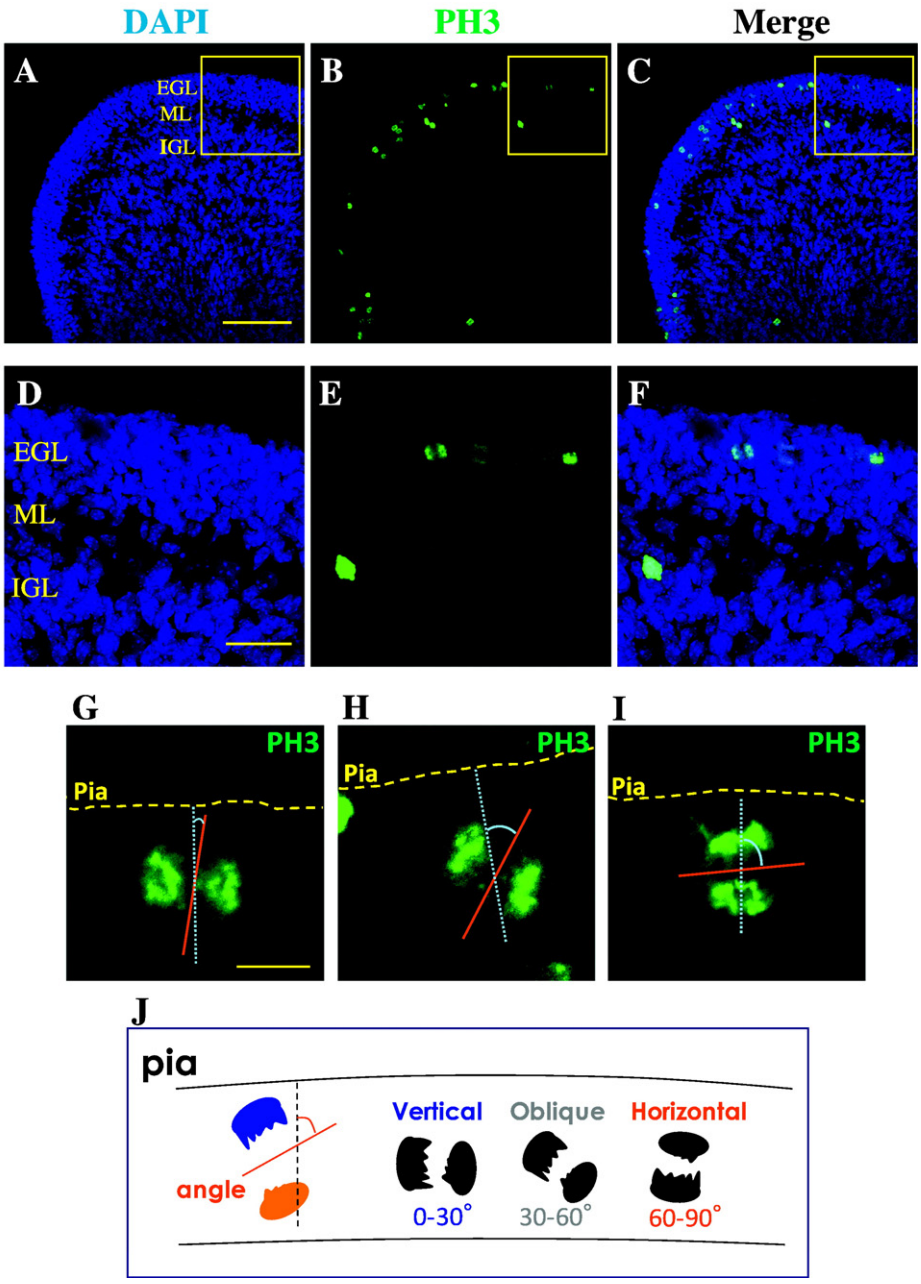

Fig. 1. PH3 staining and categorization of cleavage plane angles of dividing GCPs. (A–C) Immunostaining of PH3 (green) and nuclei (blue) in sagittal sections from P6 cerebella. Scale bar 100 μm. (D–F) High-magnification images of boxed area in A–C. Scale bar 30 μm. Representative dividing GCPs that are categorized as (G) vertical, (H) oblique and (I) horizontal divisions. (J) Schematic showing cell division categories. The angle to the pial surface plane of daughter cell cleavage angles was measured with ImageJ.

cultured cerebellar slices resulted in randomized orientation of GCP division, suggesting that SHH signaling regulates the direction of GCPs in the EGL. Our findings provide fundamental data to understanding GCP/GC development.

2. Materials and methods

2.1. Animals

All animal experiments in this study have been approved by the Animal Care and Use Committee of the National Institute of Neuroscience, Japan. Embryos and neonatal ICR mouse were fixed with 4% PFA and embedded sagittally or coronally with O.C.T. compound (Sakura Finetek). Frozen brains were sectioned into 16 μm slices with cryostat (Leica Biosystems).

2.2. Immunohistochemistry

Detailed protocols were described previously (Seto et al., 2014). In short, cerebellar sections were incubated at room temperature with 1% donkey serum containing 0.2% PBST (blocking solution) for one hour. After blocking solutions were removed, anti-phosphohistone H3

Fig. 2. Analysis of the orientation of GCP division during development in sagittal section. PH3-labelled cells in sagittal sections of (A) E16.5, (B) P0, (C) P3, (D) P6, (E) P9, (F) P12 and (G) P15 cerebellum. H, O, and V, indicate horizontal, oblique and vertical division of GCPs, respectively. Dotted lines indicate the borders of EGL. Scale bar 30 μm. (H–N) Distributions of angles of E16.5–P15 dividing GCPs were plotted on pi-chart by 15°. Over 150 cells from 3 individual mice along the midline vermis were recorded. (O) Graph shows dynamic changes of the rates of horizontal, oblique and vertical divisions in the sagittal sections during cerebellar development. Error bars represent S.E.M.

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