Stem Cell Reports
Article
OPEN ACCESS

Cell Division Mode Change Mediates the Regulation of Cerebellar Granule Neurogenesis Controlled by the Sonic Hedgehog Signaling

Rong Yang,[1] Minglei Wang,[1] Jia Wang,[1] Xingxu Huang,[2] Ru Yang,[1,*] and Wei-Qiang Gao[1,3,*]
[1]State Key Laboratory of Oncogenes and Related Genes, Renji-MedX Stem Cell Research Center, Ren Ji Hospital, School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai 200030, China
[2]School of Life Science and Technology, ShanghaiTech University, Shanghai 200031, China
[3]Collaborative Innovation Center of Systems Biomedicine, Shanghai 200240, China
*Correspondence: yangru@yahoo.com (R.Y.), gao.weiqiang@sjtu.edu.cn (W.-Q.G.)
http://dx.doi.org/10.1016/j.stemcr.2015.09.019

SUMMARY

Symmetric and asymmetric divisions are important for self-renewal and differentiation of stem cells during neurogenesis. Although cerebellar granule neurogenesis is controlled by sonic hedgehog (SHH) signaling, whether and how this process is mediated by regulation of cell division modes have not been determined. Here, using time-lapse imaging and cell culture from neuronal progenitor-specific and differentiated neuron-specific reporter mouse lines (*Math1-GFP* and *Dcx-DsRed*) and *Patched*$^{+/-}$ mice in which SHH signaling is activated, we find evidence for the existence of symmetric and asymmetric divisions that are closely associated with progenitor proliferation and differentiation. While activation of the SHH pathway enhances symmetric progenitor cell divisions, blockade of the SHH pathway reverses the cell division mode change in *Math1-GFP;Dcx-DsRed;Patched*$^{+/-}$ mice by promoting asymmetric divisions or terminal neuronal symmetric divisions. Thus, cell division mode change mediates the regulation of cerebellar granule neurogenesis controlled by SHH signaling.

INTRODUCTION

Cell division mode studies have been mainly carried out in the embryogenesis of the invertebrates such as *Drosophila* and *C. elegans* where self-renewal and cell fate specification of stem cells are accurately controlled (Bertrand and Hobert, 2009; Knoblich, 2008; Li et al., 2013b). Cell division modes include both symmetric and asymmetric divisions. The former can be further categorized as non-terminal symmetric divisions and terminal symmetric divisions. By non-terminal symmetric divisions, progenitor cells can generate two progenitor cells, expanding the progenitor pool. By terminal symmetric divisions, a progenitor cell produces two differentiated neurons, thus gradually depleting the progenitor pool. However, by asymmetric progenitor divisions, a progenitor generates one progenitor cell and one differentiated neuron, maintaining the progenitor pool and producing the differentiated progeny. Recent studies have shown that these cell division modes are also observed in the developing mammalian neocortex (Gao et al., 2014; Noctor et al., 2004; Wang et al., 2009; Zhong, 2008; Zhong and Chia, 2008; Zhou et al., 2007). However, whether a similar mechanism occurs elsewhere in the mammalian CNS, such as the cerebellum, is poorly studied.

Cerebellar development exhibits a lot of unique features that are different from cerebral neurogenesis. While most cerebral neurons are generated from the ventricle zone residing in the deep layer of cortex, cerebellar granule cells are produced in the outside of the cerebellum (Hatten and Heintz, 1995). In addition, unlike most cerebral neuronal stem cells, cerebellar granule neuronal progenitors (GNPs) are highly proliferative cells that remain active in mitosis in the external granule layer (EGL) even after birth. During the first 2–3 postnatal weeks, GNPs differentiate, exit the cell cycle, and migrate inward to form the internal granule layer, with EGL disappearing in parallel gradually. The spatiotemporal steps of proliferation and differentiation of GNPs have been described in our previous work (Gao et al., 1991; Gao and Hatten, 1993). Recent studies have demonstrated that sonic hedgehog (SHH) secreted by Purkinje cells can regulate the proliferation of GNPs (Wechsler-Reya and Scott, 1999). When treated with recombinant SHH, GNPs can be induced to undergo a long-lasting proliferation, preventing them from differentiation. However, whether such effects by SHH are mediated by changes of symmetric and asymmetric divisions of GNPs has not been studied.

In this study, we performed cell division mode analyses using various GNP-specific and differentiated granule neuron (GN)-specific reporter mice and carried out fluorescence confocal or multi-photon microscopy and time-lapse image acquisition experiments in cell cultures, as well as in freshly dissected whole-mount cerebella (ex vivo). We found evidence for the existence of non-terminal symmetric divisions, terminal symmetric divisions, and asymmetric divisions by GNPs. More importantly, activation of the SHH pathway in *Patched*$^{+/-}$ mutant mice increased proliferation and the number of GNPs by enhancing non-terminal symmetric cell divisions. On the contrary,

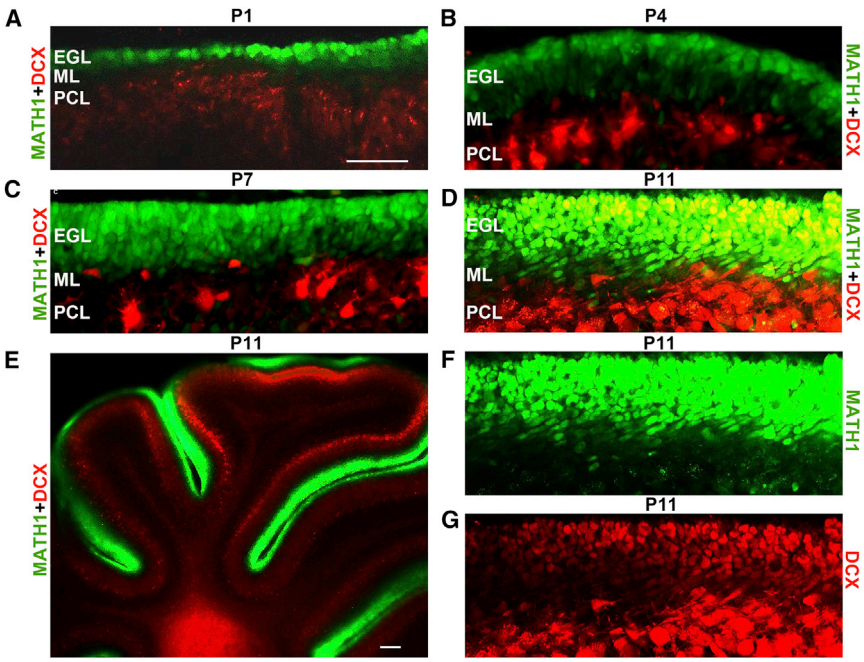

Figure 1. Characterization of *Math1-GFP;Dcx-DsRed* Mice

Math1-GFP;Dcx-DsRed mice strains were developed by crossing transgenic *Math1-GFP* and transgenic *Dcx-DsRed* mice. Cerebellar slices shown in P1 (postnatal day 1) (A), P4 (B), P7 (C), and P11 (D–G) indicate that progenitor cells are present in EGL at P1, increase in numbers at P4 and P7, and reach the number peak at P11, which is consistent with previous reports. MATH1 refers to cerebellar progenitor cells, and DCX is a biomarker of differentiated cells. MATH1 and DCX co-expressing cells represent a cell population at an intermediate stage of differentiation, undergoing a switch from MATH1-expressing cells to DCX-expressing cells, which are termed intermediate cells. See also Figure S1.

Scale bars, 60 μm for (A–D), (F), and (G) and 200 μm for (E).

administration of a small molecule inhibitor of the SHH pathway elicited the opposite effect, shifting the cell divisions to more asymmetric divisions. Thus, cell division mode changes mediate the regulation of GN neurogenesis governed by SHH signaling.

RESULTS

Math1-GFP;Dcx-DsRed Mice Are a Useful Model to Study Neurogenesis in the Cerebellum

To investigate cell division modes, cell fate specification, and neuronal differentiation in the cerebellum, we first introduced *Math1-GFP* and *Dcx-DsRed* mice from the Jackson Laboratory or Novartis, and then generated *Math1-GFP;Dcx-DsRed* mice by intercrossing the two lines. While MATH1 is a marker for cerebellar granule progenitors (Ben-Arie et al., 1997; Lumpkin et al., 2003), DCX (doublecortin) is specifically expressed by the early differentiated cerebellar GNs that just come out of the terminal mitosis (Francis et al., 1999; Gleeson et al., 1999; Wang et al., 2007). With the *Math1-GFP;Dcx-DsRed* mice, both progenitors and differentiated GNs could easily been observed in vivo or in dissociated cell cultures. An example of the well-labeled cells at postnatal day 11 (P11) is shown in Figure 1. While green fluorescent staining represented progenitor cells, red fluorescent labeling showed differentiated GNs. Yellow fluorescent staining displayed cells that were at an intermediate stage before acquisition of the differentiated neuronal fate. Therefore, the *Math1-GFP;Dcx-DsRed* mouse line provides an informative system for studying temporal GN neurogenesis, including self-renewal, cell fate specification, and neuronal differentiation.

GNPs Switch Gradually from MATH1-Expressing to DCX-Expressing Cells

To directly visualize the GNPs and differentiated GNs, we performed multi-photon microscopy of sagittal cerebellar sections prepared from *Math1-GFP;Dcx-DsRed* double reporter mice at various developmental stages ranging from P1 to P11. We observed a stepwise pattern of cerebellar neurogenesis during development. As shown in Figure 1, MATH1-positive cells were initially detectable in the EGL at P1 (Figure 1A), increased in numbers at P4 and P7 (Figures 1B and 1C), and reached a peak at P11 (Figures 1D–1G). In contrast, DCX-positive cells did not appear in the EGL until P7 and P11. In particular, many yellow fluorescent cells, a cell population co-expressing MATH1 and DCX, emerged at P11 (Figures 1D and 1E). This population appeared to represent the cells undergoing a switch from MATH1-expressing cells to DCX-expressing cells. We termed the yellow subpopulation intermediate cells; these have not been described previously.

Morphologically, we found that MATH1-GFP-positive GNPs looked like epithelioid cells based on the green and red fluorescence of *Math1-GFP;Dcx-DsRed* mice in vivo (Figure S1A). In contrast, DCX-DSRED-positive GNs appeared more like polyhedral cells with processes (Ryder and Cepko, 1994) (Figure S1B), and MATH1-GFP/DCX-DSRED double-positive intermediate cells were at the stage of changing from epithelioid cells to polyhedral cells (Figures