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Cerebellar stem cells do not produce neurons and astrocytes in adult mouse

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ABSTRACT

Although previous studies implied that cerebellar stem cells exist in some adult mammals, little is known about whether these stem cells can produce new neurons and astrocytes. In this study by bromodeoxyuridine (BrdU) intraperitoneal (i.p.) injection, we found that there are abundant $BrdU^+$ cells in adult mouse cerebellum, and their quantity and density decreases significantly over time. We also found cell proliferation rate is diversified in different cerebellar regions. Among these $BrdU^+$ cells, very few are $mash1^+$ or $nestin^+$ stem cells, and the vast majority of cerebellar stem cells are quiescent. Data obtained by *in vivo* retrovirus injection indicate that stem cells do not produce neurons and astrocytes in adult mouse cerebellum. Instead, some cells labeled by retrovirus are $Iba1^+$ microglia. These results indicate that very few stem cells exist in adult mouse cerebellum, and none of these stem cells contribute to neurogenesis and astrogenesis under physiological condition.

1. Introduction

In mammalian brain, adult neurogenesis is thought to take place in highly restricted sites-the forebrain subventricular zone (SVZ) and the hippocampal subgranular zone (SGZ) [1–3]. Normality of specific brain functions such as learning, memory, olfaction, and mood modulation requires participation of adult-born neurons [4,5]. Recent years, the occurrence of "incomplete" neurogenic and gliogenic processes had been found in some non-neurogenic regions of adult mammalian brain, such as hypothalamus [6,7], brainstem [8], striatum [9], amygdala [10,11]. In terms of mammalian cerebellum, it has been long believed that neurogenesis is limited to early postnatal stage, and no neurogenesis exists in adult [11–13]. However, recent studies on New Zealand white rabbit showed that cerebellar neurogenesis can also be maintained into adulthood [14–17]. Moreover, a research in cat cerebellum suggested neurogenesis of unipolar brush cells (UBCs), one subtype of cerebellar interneurons, may continue for several postnatal months [18]. In contrast, other studies revealed that neurogenesis is complete by about 3–4 postnatal weeks in cat cerebellum [19,20]. Of note, a recent study found the expression of polysialylated neural cell adhesion molecule (PSA-NCAM) and doublecortin (DCX), markers for newborn and migrating immature neurons, in UBCs in adult rat cerebellum [21].

In this study, we directly inspected whether neurogenesis takes place in adult mouse cerebellum by a combination of BrdU i.p. injection, EGFP retrovirus *in vivo* delivery and immunohistochemical analysis. We identified that very few stem cells exist in adult mouse cerebellum, and none of these stem cells contribute to neurogenesis and astrogenesis under physiological condition.

2. Materials and methods

2.1. Bromodeoxyuridine labeling

All experiments and animal care were operated under the guidelines of Fudan University Shanghai Medical College. 2-month-old and 9-month-old CD-1 mice received intraperitoneal injection of bromodeoxyuridine (BrdU, 25 mg/kg body weight, Sigma) following the protocol described in Results.

Mice were intracardially perfused with cold phosphate-buffered saline (PBS, pH 7.4) and cold 4% paraformaldehyde (PFA) in PBS. The brains were fixed in 4% PFA overnight, washed in PBS, and coronally sectioned at 60 μm by vibratome (VT1000S, Leica). Sections were pretreated by 2 N HCl for one hour at 37 °C, followed

Abbreviations: BrdU, bromodeoxyuridine; i.p., intraperitoneal; ML, the molecular layer; GL+PL, the granule cell layer and Purkinje cell layer; WM, white matter; DCN, the deep cerebellar nuclei; UBCs, unipolar brush cells; PSA-NCAM, polysialylated neural cell adhesion molecule; DCX, doublecortin; EGFP, enhanced green fluorescent protein.

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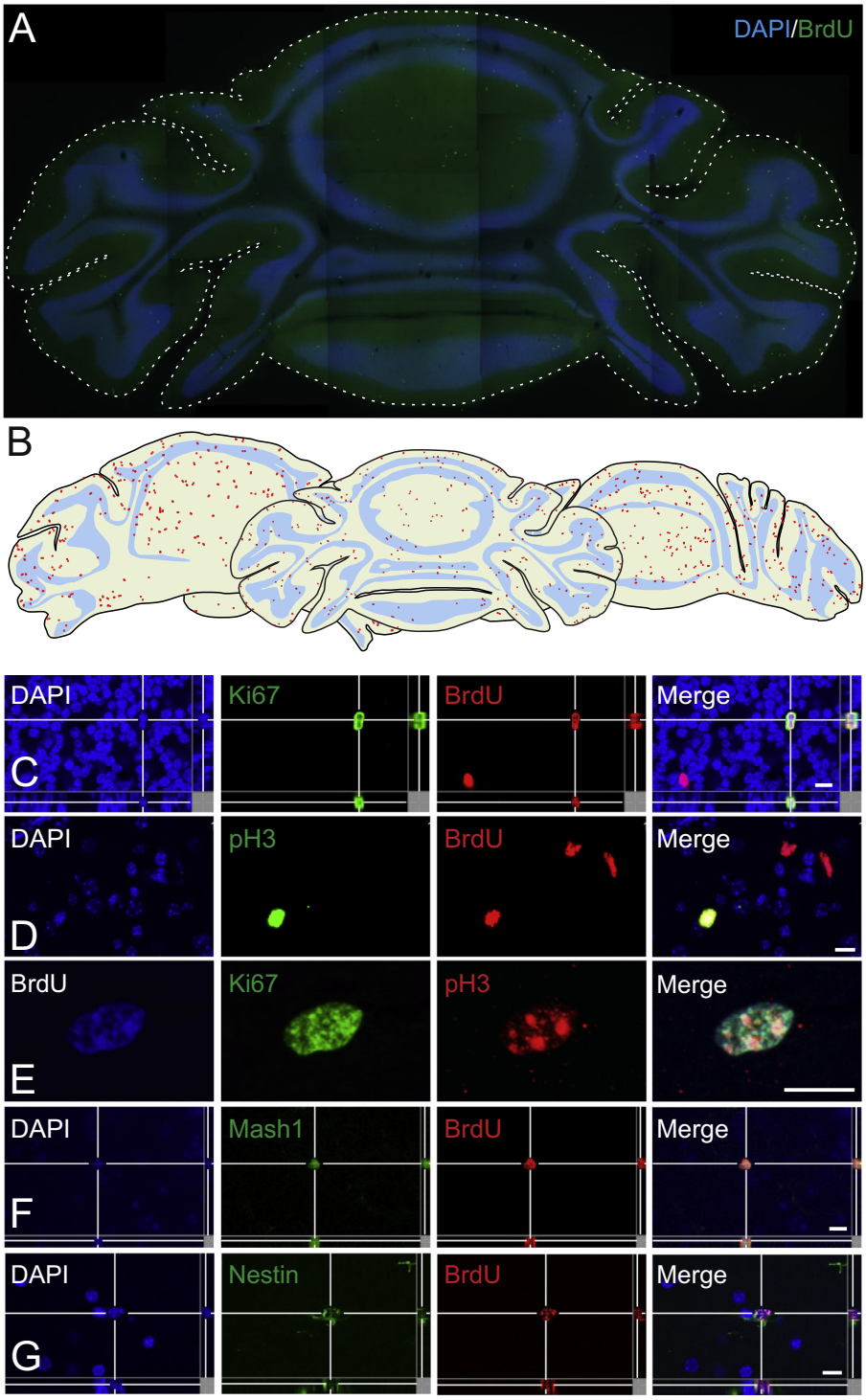

Fig. 1. Abundant BrdU$^+$ cells distributed in adult mouse cerebellum, but very few of them are stem cells. (A) Representative image of adult mouse cerebellum immunostained with antibodies against BrdU and DAPI after series of BrdU i.p. injections. (B) Plots illustrating the distribution of BrdU$^+$ cells in cerebellum. (C and D) Representative images showing colocalization of BrdU with proliferating cell markers Ki67 and pH3, respectively. (E) Tri-labeling between BrdU, Ki67 and pH3. (F and G) Representative image showing colocalization of BrdU with stem cell marker mash1 and nestin. Scale bar: 10 μm.

by 0.1 M boric acid (pH 8.44) for 30 min. Primary antibodies in donkey serum solution (10% donkey serum, 0.5% Triton-X-100) were applied for 48 h at 4 °C, and then appropriate secondary antibodies were applied for 2 h in the dark at room temperature. Sections were mounted and visualized under confocal laser scanning microscope (FV1000, Olympus) or epifluorescence microscope (BX41, Olympus) and processed by Imaris (Bitplane), Neurolucida (Microbrightfield) and Adobe Photoshop CS5 (Adobe system). The following primary antibodies were used: rat anti-BrdU (1:100, AbD), rabbit anti-phosphorylated histone H3 (pH3, 1:500, Millipore), mouse anti-Ki67 (1:500, DakoCytomation), mouse anti-mash1 (1:400, BD pharmingen), mouse anti-nestin (1:100, Developmental Studies Hybridoma Bank), mouse anti-parvabumin (PV, 1:500, Millipore), mouse anti-calbindin (CB, 1:5000, Swant), goat anti-calretinin (CR, 1:500, Millipore), mouse anti-NeuN (1:500, Millipore). The secondary antibodies: goat anti-rat (Alexa

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