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Regulation by commensal bacteria of neurogenesis in the subventricular zone of adult mouse brain

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ABSTRACT

In the mouse olfactory bulb (OB), interneurons such as granule cells and periglomerular cells are continuously replaced by adult-born neurons, which are generated in the subventricular zone (SVZ) of the brain. We have now investigated the role of commensal bacteria in regulation of such neuronal cell turnover in the adult mouse brain. Administration of mixture of antibiotics to specific pathogen-free (SPF) mice markedly attenuated the incorporation of bromodeoxyuridine (BrdU) into the SVZ cells. The treatment with antibiotics also reduced newly generated BrdU-positive neurons in the mouse OB. In addition, the incorporation of BrdU into the SVZ cells of germ-free (GF) mice was markedly reduced compared to that apparent for SPF mice. In contrast, the reduced incorporation of BrdU into the SVZ cells of GF mice was recovered by their co-housing with SPF mice, suggesting that commensal bacteria promote the incorporation of BrdU into the SVZ cells. Finally, we found that administration of ampicillin markedly attenuated the incorporation of BrdU into the SVZ cells of SPF mice. Our results thus suggest that ampicillin-sensitive commensal bacteria regulate the neurogenesis in the SVZ of adult mouse brain.

1. Introduction

Interneurons, such as granule cells and periglomerular cells, of the mammalian olfactory bulb (OB) are generated continuously from neural stem cells that reside in the subventricular zone (SVZ) of the lateral ventricle (LV) throughout adulthood [1]. A subpopulation of astrocytes, called type B1 cells, is thought to serve as the neural stem cells and to generate proliferating progeny, called type C cells, in the SVZ. Type C cells subsequently differentiate into neuroblasts, called type A cells [1]. Type A cells divide and migrate along the rostral migratory stream into the OB, where they differentiate into mature interneurons. The continuous production of new interneurons is thus balanced by the elimination of older interneurons, resulting in a rapid turnover of interneurons in the OB.

Such rapid turnover of interneurons in the OB is thought to be largely dependent on proliferation and migration of their progenitor cells in the SVZ. A previous study showed that the canonical Wnt pathway that stabilizes β-catenin promotes proliferation of both type B1 and type C cells in the SVZ [2]. Moreover, the non-canonical Wnt pathway (planar cell polarity pathway) is known to regulate the proliferation, migration and maturation of newly generated neurons [3–5]. In contrast, the Notch pathway is thought to be important for maintaining the type B1 cells [6]. In addition, receptors for epidermal growth factor and fibroblast growth factor are expressed in the SVZ, and ablation of their ligands reduces neurogenesis in the SVZ [7,8], suggesting that these growth factors likely regulate neurogenesis in the SVZ.

Gut commensal bacteria have recently been demonstrated to play important roles in the homeostatic regulation of neuronal cells in the brain. Commensal bacteria are shown to regulate blood-brain barrier integrity, microglial maturation and myelination [9]. Furthermore, commensal bacteria are also shown to regulate the expression of neurotrophins, neurotransmitters and their receptors, and modulate behaviors [9]. In addition to the SVZ of the LV, adult neurogenesis occurs in the subgranular zone (SGZ) of the

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Abbreviations

ABPC	ampicillin
Abx	antibiotic-treated SPF
BrdU	bromodeoxyuridine
co-GF	co-housed GF
Ctrl	control SPF
DAPI	4′, 6-diamidino-2-phenylindole
DCX	doublecortin
GCL	granule cell layer
GF	germ-free
GFAP	glial fibrillary acidic protein
IECs	intestinal epithelial cells
IPL	internal plexiform layer
LV	lateral ventricle
mAb	monoclonal antibody
Mash1	mammalian achaete-scute homolog 1
MCL	mitral cell layer
MNZ	metronidazole
NeuN	neuronal nuclear antigen
NM	neomycin
OB	olfactory bulb
SE	standard error
SGZ	subgranular zone
SPF	specific pathogen-free
SVZ	subventricular zone
VCM	vancomycin

hippocampal dentate gyrus [10]. Commensal bacteria are also suggested to regulate adult hippocampal neurogenesis [11]. Thus, we have now examined the role of commensal bacteria in regulation of neurogenesis at SVZ of the adult mouse brain.

2. Materials and methods

2.1. Mice

C57BL/6J and C57BL/6N male mice were obtained from CLEA Japan (Tokyo, Japan). These mice were maintained at the Institute for Experimental Animals at Kobe University Graduate School of Medicine under the specific pathogen-free (SPF) condition. Germ-free (GF) mice (C57BL/6N background) were obtained from CLEA Japan (Tokyo, Japan). For Reconstitution with normal commensal bacteria in GF mice, GF mice were co-housed with SPF mice from 4 to 9 week-old under the SPF condition. All animal experiments were performed according to Kobe University animal experimentation regulations.

2.2. Antibodies and reagents

A rat monoclonal antibody (mAb) to bromodeoxyuridine (BrdU) (#ab6326) was obtained from Abcam (Cambridge, MA). A mouse mAb to glial fibrillary acidic protein (GFAP) (#G3893) was obtained from Sigma-Aldrich (St. Louis, MO). A mouse mAb to mammalian achaete-scute homolog 1 (Mash1) (#556604) was obtained from BD Biosciences (San Diego, CA). A mouse mAb to doublecortin (#sc-271390) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). A mouse mAb to NeuN (#MAB377) was obtained from Merck Millipore (Billerica, MA). Secondary antibodies labeled with Cy3 or Alexa488 for immunofluorescence analysis were obtained from Jackson ImmunoResearch (West Grove, PA) and ThermoFisher (Waltham, MA), respectively. Ampicillin, vancomycin, and metronidazole were obtained from Wako (Osaka, Japan). Neomycin sulfate and 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Nacalai Tesque (Kyoto, Japan), and BrdU was from Sigma-Aldrich.

2.3. Antibiotic treatment

An antibiotic treatment to mice was performed as previously described [12,13] but with a slight modification. In brief, mice were treated orally with an antibiotic cocktail (ampicillin, metronidazole, and neomycin each at 1 g/l, and vancomycin at 0.5 g/l) or with each antibiotic alone in drinking water from 4 to 9 week-old under the SPF condition.

2.4. BrdU incorporation

For analysis of the BrdU-incorporation into the SVZ, mice were injected intraperitoneally with BrdU (300 mg/kg). After 4, 8 or 24 h, brain samples for immunofluorescence analysis were prepared. For analysis of the number of BrdU-positive neurons in the OB, mice were injected intraperitoneally with BrdU (300 mg/kg), followed by provision of 1 mg/ml BrdU in drinking water for 14 days.

2.5. Immunofluorescence analysis

Preparation of the coronal sections of the SVZ was performed as previously described [14] but with a slight modification. In brief, 0–1400 μm rostral to the convergence of the anterior commissure was defined as containing the SVZ. Ten frozen sections with a thickness of 10 μm, each 150 μm apart (1:15 series), were prepared.

Coronal sections of the SVZ or the OB were subjected to immunofluorescence analysis with primary antibodies and fluorescent dye-labeled secondary antibodies with or without DAPI as described previously [15]. Labeled cells were counted under a fluorescence microscope (BX51; Olympus, Tokyo, Japan) or a laser scanning confocal microscope (LSM700; Carl Zeiss, Oberkochen, Germany), or analyzed with the use of ImageJ software (NIH).

For detection of BrdU, frozen sections were incubated for 30 min at 37 °C with 1.5 N HCl, washed with 0.1 M borate buffer (pH 8.5), and then subjected to immunofluorescence analysis as described above.

2.6. Statistical analysis

Data are presented as means ± standard error (SE). Unpaired, two-tailed Student's t-tests were used to compare between 2 groups. One-way ANOVA followed by Tukey's tests were used to compare among 3 groups. Results were analyzed using GraphPad Prism software version 6.0 (GraphPad Software). A P value of <0.05 was considered statistically significant.

3. Results

3.1. Marked reduction of the BrdU-incorporation into the SVZ of antibiotic-treated SPF mice

To determine the effect of antibiotics in adult neurogenesis in the SVZ of the mouse brain, we first treated 4-week-old SPF mice with an antibiotic cocktail [ampicillin, vancomycin, metronidazole, and neomycin] in drinking water for 5 weeks. Such treatment was previously demonstrated to efficiently deplete commensal bacteria

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