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Neural stem and progenitor cells in the aged subependyma are activated by the young niche

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ABSTRACT

Previous studies have demonstrated an age related decline in the size of the neural stem cell (NSC) pool and a decrease in neural progenitor cell proliferation, however, the mechanisms underlying these changes are unclear. In contrast to previous reports, we report that the numbers of NSCs is unchanged in the old age subependyma and the apparent loss is because of reduced proliferative potential in the aged stem cell niche. Transplantation studies reveal that the proliferation kinetics and migratory behavior of neural precursor cells are dependent on the age of the host animal and independent of the age of the donor cells suggesting that young and old age neural precursors are not intrinsically different. Factors from the young stem cell niche rescue the numbers of NSC colonies derived from old age subependyma and enhance progenitor cell proliferation in vivo in old age mice. Finally, we report a loss of Wnt signaling in the old age stem cell niche that underlies the lack of expansion of the NSC pool after stroke.

1. Introduction

There is a growing body of evidence demonstrating that endogenous adult stem cells and their niche change during aging (Ahlenius et al., 2009; Conboy et al., 2005; Geiger and Van Zant, 2002; Hattiangady and Shetty, 2008; Suchitra and Thomas, 2008; Warren and Rossi, 2009). In the nervous system, a decrease in stem and progenitor cell proliferation has been reported in the adult stem cell niche, the subependyma (SE) lining the forebrain lateral ventricles, resulting in reduced olfactory bulb (OB) neurogenesis and a loss of fine odor discrimination (Enwere et al., 2004; Tropepe et al., 1997). Concomitant with the decreased neurogenesis, numerous reports reveal that the numbers of adult neural stem cells decline into old age (Ahlenius et al., 2009; Enwere et al., 2004) as revealed by a decrease in colony formation in vitro. Because the in vitro colony forming assays are inherently dependent on cell proliferation, these studies could not distinguish a decrease in stem cell number from a lack of stem cell and/or progenitor cell proliferation.

Whether these changes observed with aging are the result of precursor cell intrinsic changes or changes in the stem cell niche has not been established. Studies of neurogenesis in the hippocampus have revealed that extrinsic cues from the environment, such as changes in growth factor signaling (Shetty et al., 2005), morphogen levels (Ahlenius et al., 2009; Okamoto et al., 2011), and corticosteroid levels (Cameron and McKay, 1999; Montaron et al., 1999; Montaron et al., 2006) play at least a partial role in the age-related decrease in this region. Cell intrinsic changes that manifest during aging, such as changes in cell cycle regulator expression (Janzen et al., 2006; Nishino et al., 2008), cell damage (Rossi et al., 2007), and telomere shortening (Ferron et al., 2009) have been shown to impact cell proliferation. To what extent cell intrinsic and extrinsic changes underlie the reduced regeneration capacity observed in the old age brain is unclear (Buchhold et al., 2007; Lee et al., 2006; Rosen et al., 2005).

In this work we demonstrate that contrary to previous reports, the numbers of neural stem cells (NSCs) are in fact maintained in the aged SE. The apparent loss of NSCs previously reported is because of changes in the old age brain that inhibit NSC proliferation. We use in vivo studies, including cell transplantation and intraventricular infusion of factors derived from the young stem cell niche to demonstrate that changes in the neurogenic niche are responsible for the decreased progenitor cell proliferation in the old age SE. We have found a loss of endogenous Wnt signaling in the aged stem cell niche (the SE). Further, a lack of up-regulation of Wnt signaling in response to brain injury is concomitant with the inability to expand the stem cell pool after stroke in the aged brain. These findings support the hypothesis that changes in the microenvironment underlie the decreased regenerative potential of the old age brain.

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2. Methods

2.1. Animals

Young adult mice were aged 8 to 10-week-old and old age mice were 18 to 22-month-old. Mouse strains used were CD1 (Charles River, Senneville, Quebec, Canada) and YFP (Jackson Labs, Bar Harbor, ME, USA). For old age mice, the mice aged 6-month-old were ordered and housed in the animal facilities until they reached old age. Transgenic BAT-gal mice (as described previously mentioned in Maretto et al., 2003) were bred in house. C57BL/6 mice (Charles River) served as strain controls for the BAT-gal mice. All animals were housed at the University of Toronto animal facilities and maintained in accordance with the Animal Care Committee and Institutional guidelines.

2.2. Isolation and culture of SE cells

Adult mice were killed by cervical dislocation and the neural precursor cells were isolated as described previously (Chiasson et al., 1999; Morshead et al., 2003). Briefly, the periventricular region of the forebrain was dissected and placed in artificial cerebrospinal fluid. Tissue was digested with 1.33 mg/mL trypsin (Sigma, Oakville, Canada), 0.67 mg/mL hyaluronidase (Sigma), and 0.2 mg/mL kynurenic acid (Sigma) to dissociate tissue at 37 °C. Tissue was transferred to serum-free medium containing 0.7 mg/mL trypsin inhibitor (Roche, Laval, Canada) and triturated with a fire-polished pasteur pipette. To quantify the numbers of neurosphere forming cells per cell plated, the cells were cultured at the density indicated in the text in 24-well (0.5 mL/well) uncoated plates (Thermo/Nunc, Rochester, NY, USA) in serum-free media (Tropepe et al., 1999) containing epidermal growth factor (20 ng/mL; Millipore/Upstate Biotech, Billerica, MA, USA), basic fibroblast growth factor (10 ng/mL; Sigma), and heparin (2 mg/mL; Millipore/Upstate Biotech). To quantify the total number of neurosphere forming cells per SE, dissociated cells from each entire SE dissection were suspended in 250 μL of plating medium. The suspension of 50 μL was then plated into wells of a 24-well uncoated plate (0.5 mL/well) at 2500 cells/well. The neurospheres that formed were counted after 7 days in vitro and the total number was multiplied by 5 (250 μL/50 μL) to get the total number of neurospheres per brain. For single neurosphere passaging, individual neurospheres were collected in a 200-mL pipette tip, transferred to a 500-μL eppendorf tube containing 200 μL plating medium, triturated and plated in a final volume of 500 μL medium in a 24-well plate. ZVAD (R&D systems, Minneapolis, MN, USA), sFPR2 (R&D systems), or SB216763 (Sigma) were added to the media as indicated in the text. Conditioned media (CM) were generated by plating young or old age SE cells at 40 cells/μL in standard neurosphere media as mentioned previously in 6-well plates. After 24 hours, the media were collected by spinning down the cells, collecting the supernatant, and filtering (0.45 μm syringe filter) (Acrodisc by Pall, Port Washington, NY, USA). The CM was used immediately upon collection.

2.3. Transplants

Cells were isolated from YFP mice as previously mentioned and cultured for 7 days to form neurospheres. Neurospheres were dissociated by light trituration and resuspended in 15 μL of artificial cerebrospinal fluid. Animals were anesthetized with isofluorane (1%) and 150,000 cells were sterotaxically transplanted at anterior-posterior (AP)-0 mm and lateral-1.4 mm, relative to bregma and 2.2 mm below the surface of the skull.

2.4. Intraventricular infusions

Animals (young or aged) were anesthetized with isofluorane (1%). SB216763 (200 μM) was intraventricularly infused unilaterally using an osmotic minipump (model 1007D, Alzet, Cupertino, CA, USA) placed subcutaneously over the shoulder. The cannula was placed onto the surface of the skull and penetrated the brain at A/P-0 mm, lateral 0.7 mm relative to bregma and 2.5 mm below the surface of the skull. Vehicle infusions (DMSO [dimethyl sulfoxide] for SB216763 experiments; control media (media that had been incubated alongside the CM, but that had no cells added to it, for CM experiments) served as controls. Only the ipsilateral hemisphere was used for subsequent analysis (neurosphere assay or staining). Some animals received intraperitoneal injections of bromodeoxyuridine (BrdU; 65 mg/kg in saline; Sigma) as indicated in the text.

2.5. Histochemistry

Animals were overdosed with pentobarbital and perfused transcardially with 2% paraformaldehyde. Brains were removed and cryoprotected in 20% sucrose overnight before cryosectioning (15 μm thick coronal sections). For X-Gal staining, the sections were washed and incubated in X-gal solution for 1 hour as described (Lobe et al., 1999). For BrdU staining sections were incubated in 1 N HCl at 60 °C for 30 minutes before incubation in rat anti-BrdU antibody (1:100; Seralab, West Sussex, UK) at 4 °C, followed by TRITC donkey anti-rat antibody (1:200; Jackson Immunoresearch, West Grove, PA, USA). The total numbers of X-Gal or BrdU labeled cells were counted surrounding the lateral ventricles between the crossing of the genu of the corpus callosum and the crossing of the anterior commissure. For transplant studies, sagittal sections were examined using a GFP antibody that cross reacts with YFP to visualize cells: rabbit anti-GFP (1:500; a11122, Life Technologies/Molecular Probes, Burlington, Canada) and mouse anti-NeuN (1:100, MAB377, Millipore, MA, USA) with Alexa Fluor (1:400; Life Technologies/Invitrogen, Burlington, Canada) secondary antibodies. For differentiated cells, the following antibodies were used for immunostaining: rabbit anti-GFAP (1:500; Sigma), mouse anti-βIII-tubulin (1:500; Sigma), mouse anti-O4 (1:75, Millipore). Alexa secondary antibodies (1:400; Life Technologies/Invitrogen).

2.6. Stroke

Animals were anesthetized with isofluorane (1%). A drill was used to cut a rectangular hole in the frontal and parietal bones running from 0.5 mm posterior to 2.5 mm anterior to bregma and 0.5 mm to 3.5 mm lateral from the midline. The dura was removed and the pia and attached blood vessels were wiped from the cortical surface using a sterile saline soaked cotton swab. At day 7 post-stroke, the brains were perfused as described previously.

2.7. Statistics

Used t test for comparison of pairs and 1-way analysis of variance for group comparisons, with $p = 0.05$ as cutoff for significance. The test used for each experiment is indicated in the figure legends.

3. Results

3.1. Neural stem cell colonies from old age mice have similar properties to those derived from young mice

To examine neural precursor cell behavior we used the neurosphere assay. Briefly, SE cells are dissected from the adult brain (young and aged) and plated in growth factor conditions, in which