Neurobiology of Aging 34 (2013) 1712.e15–1712.e21

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Effects of age and strain on cell proliferation in the mouse rostral migratory stream

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ARTICLE INFO

Article history:
Received 14 July 2012
Received in revised form 21 December 2012
Accepted 24 December 2012
Available online 28 January 2013

Keywords:
Aging
Neurogenesis
Rostral migratory stream
Adult neural progenitor cells
Cell proliferation
Bromodeoxyuridine
Inbred mice
Quantitative trait loci

ABSTRACT

The number of neural progenitor cells (NPCs) decreases with advancing age, and the mechanisms responsible for this decline is unclear. Here, we demonstrate the importance of genetics as a modulator for the age-related decline in NPCs. We systematically quantified the number of proliferating NPCs in the rostral migratory stream, the rostral extension of the subventricular zone, in 9 inbred mouse strains from 3 to 18 months of age. A striking negative impact of age and significant interstrain differences in the number of NPCs was detected at 3 and 12 months of age. Extended proliferative profiles of C57BL/6J and DBA/2J from 3 to 24 months of age revealed differential dynamics of the age-related decline in NPCs. Statistically significant interaction effects for age and strain were detected over the 3- to 7-month period. Strain differences were mapped to several genetic loci suggesting complex genetic control of NPC proliferation at different ages. Furthermore, correlational analyses revealed the differential regulation of cell proliferation and genes that may underlie the proliferative deficits of NPCs in the aging brain.

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1. Introduction

Stem cell aging is regarded as one of the contributors to several degenerative conditions afflicting the elderly because it underlies the physiological decline in tissue maintenance and regenerative capacity of many organs (Rossi et al., 2008). The brain is one such organ that contains discrete populations of stem cells and their precursors (collectively referred to as neural progenitor cells [NPCs]) that continue to generate new neurons throughout life (Bedard and Parent, 2004; Eriksson et al., 1998). This biological process, also known as adult neurogenesis, predominantly takes place in the subgranular zone of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. The rostral migratory stream (RMS) is the rostral extension of the SVZ where neural precursor cells migrate and differentiate into interneurons of the olfactory bulb (Kuhn et al., 1996; Lois and Alvarez-Buylla, 1994). The continual supply of new neurons by NPCs is not only important for maintaining the integrity of specific brain regions (e.g., olfactory bulb) but has also been functionally implicated in learning and memory (Gheusi et al., 2000; Imayoshi et al., 2008; van Praag et al., 2002).

A wide range of factors have been shown to regulate adult neurogenesis (reviewed by Ming and Song, 2011). Of these, age is identified as a negative regulator of neurogenesis where dramatic reduction in the rate of neuronal production has been reported with advancing age (Bouab et al., 2011; Enwere et al., 2004; Kuhn et al., 1996; Lazarov et al., 2010; Tropepe et al., 1997). Concomitant with this age-related decrease in neurogenesis are cognitive impairment and memory decline (Enwere et al., 2004; Lazarov et al., 2010; Villeda et al., 2011). The precipitous drop in neuronal production is largely attributable to the reduced rate of NPC proliferation (Kuhn et al., 1996; Luo et al., 2006; Maslov et al., 2004), and the cause of the decline remains to be elucidated. Given that aging is also a risk factor for several neurologic disorders and brain injuries (e.g., Alzheimer's disease and cerebral ischemia), the manipulation of NPCs may be an effective means of compensating neuronal loss in these pathophysiological conditions. However, our understanding of the molecular changes associated with the age-related decline in neurogenesis is sparse. The development of regenerative strategies will require the characterization of neurogenesis during senescence and the elucidation of mechanisms underlying the age-related NPC decline.

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0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.12.021

In addition to age, genetic background has also been shown to significantly influence neurogenesis in young adult mice (Kempermann and Gage, 2002; Kempermann et al., 2006; Poon et al., 2010). Our earlier work detected marked strain differences in NPC proliferation in the RMS of C57BL/6J and A/J and their recombinant inbred (RI) strains, the AXB/BXAs (Poon et al., 2010). Cell proliferation in the RMS behaves as a quantitative trait and the heritability of this trait was estimated to be 0.53, suggesting an important genetic component to NPC proliferation (Poon et al., 2010). The rich phenotypic differences allowed us to subsequently perform genome-wide mapping and discovered a significant quantitative trait locus (QTL) on chromosome 11 that affects cell proliferation in the adult RMS. The success of our QTL mapping demonstrates the feasibility of using both phenotypic and genetic diversities present in the inbred strains as means to identify genes potentially important for modulating adult neurogenesis.

It is currently unknown whether the prominent genetic background effect observed in young adults persists into later life. The goal of this study is to determine age and genetic background effect on NPC proliferation through the comparison of cell proliferation in the RMS of 9 inbred mouse strains during the aging process. We systematically quantified the proliferative population in the RMS 1 h after an injection of the thymidine analog bromodeoxyuridine (BrdU). Our longitudinal study from 3 to 24 months of age confirmed the negative impact of age on NPC proliferation in the RMS. Interstrain comparisons and genome-wide association mapping allowed us to further conclude that neural proliferation in the RMS is modulated by the genetic differences present in mouse strains. The background effect is highest in young adults and wanes with advancing age. In addition, a statistically significant effect of strain $\times$ age interaction was found, and this was supported by the differential dynamics in the age-dependent decline displayed by the 2 commonly used inbred mouse strains, C57BL/6J and DBA/2J. The significant interaction effect points to the presence of genes whose expressions are dependent on genetic background and aging. We further demonstrated the effectiveness of the combined use of genome-wide association mapping with correlation analyses against existing phenotypic and expression data sets to identify candidate genes that may be involved in the age-related decline in adult neurogenesis.

2. Methods

2.1. Animals

Nine inbred strains, C57BL/6J, FVB/NJ, A/J, BALB/cByJ, C3H/HeJ, CBA/J, DBA/2J, 129S1/SvImJ, and 129X1/SvJ, were used for these experiments. These strains were selected for the following reasons. (1) They are broadly distributed on the mouse phylogenetic tree (Petkov et al., 2004) and are expected to be genetically diverse. (2) All these strains have been haplotyped (National Institute of Environmental Health Sciences/Perlegen), and most of the strains (except for 129X1/SvJ) have been sequenced (Wellcome Trust Sanger Institute—Mouse Genomes Project). These genetic information were used to pinpoint the genomic regions associated with the differences in NPC numbers. (3) The 9 strains are among the most commonly used mouse strains in research. They are considered priority strains in the Mouse Phenome Database, which currently holds 1092 phenotypic data collected from these inbred strains. This allowed us to examine correlations between our data and other traits deposited at the Mouse Phenome Database. Mice were obtained from the Jackson Laboratory and the National Institutes of Aging. A total of fifty-one 3-month-old mice (at least 2 males and 2 females for each strain), forty-nine 12-month-old mice (at least 2 males and 2 females for each strain), and thirty-nine 18-month-old mice (at least 2 males and 2 females for C57BL/6J, A/J, CBA/J, DBA/2J, 129S1/SvImJ, and 129X1/SvJ; females only for the BALB/cByJ, C3H/HeJ, and FVB/NJ) were examined and compared among the 9 strains. Additional proliferative data on 7-month and 24-month-old mice ($n = 6$ and 9, respectively) were collected for the C57BL/6J and DBA/2J inbred strains to study the dynamics of age-related changes in RMS proliferation. All experiments were conducted in accordance with the guidelines of the Canadian Council of Animal Care, and all protocols were approved by the institutional animal care committee.

2.2. BrdU labeling and detection

All mice were injected with a single dose of 50 mg BrdU/kg 1 hour before perfusion with acetic acid: 95% ethanol (1:3) as previously described (Poon et al., 2010). Brains were removed from the skull and processed for paraffin embedding. Each hemisphere was serially sectioned in the sagittal plane at 8 μm and then mounted on Superfrost/Plus slides for anti-BrdU immunohistochemistry. Briefly, sections were deparaffinized, rehydrated, and treated with 1M HCl for 30 minutes at 37 °C. Slides were incubated with mouse anti-BrdU monoclonal antibody (1:200 dilution; BD Biosciences, Mississauga, ON, Canada) overnight and then with biotinylated horse anti-mouse immunoglobulin (1:200 dilution; Vector Laboratories, Burlingame, CA, USA) for 1 hour on the next day. BrdU immunoreactivity was subsequently revealed using the VECTASTAIN Elite ABC kit (Vector Laboratories) and 3, 3′-diaminobenzidine (Sigma–Aldrich).

2.3. Quantification

Every tenth section throughout the left hemisphere was stained, and the sagittal section that contained the most intact RMS exhibiting the stereotypical trajectory of proliferating cells en route to the olfactory bulb (OB) was analyzed. BrdU-labeled cells in the RMS of this optimal section were counted under bright-field illumination and with the aid of a 20$\times$ objective (Zeiss 200M Axiovert inverted microscope equipped with Axiovision 4.6 software). RMS length was measured using NIH ImageJ (version 1.42) software. Based on these measurements, the linear density (i.e., the number of $BrdU^+$ cells per millimeter of RMS length) was calculated for each animal.

2.4. Statistical analysis

Data were anlayzed using the JMP10 statistical software (SAS Institute, Cary, NC, USA). One-way analysis of variance was performed to test whether the number of proliferative cells in the RMS varied as a function of strain. Post hoc comparisons using Tukey's Honestly Significant Difference (HSD) test were employed to determine specific strain differences in each age group. Trends of the age-related decline were assessed by segmented linear regression analysis in which the independent variable, age, is partitioned into intervals based on the proliferative data collected at different time points. General linear modeling was used to determine the contribution of strain, age, sex, and interactions on RMS proliferation. Analyses were considered significant at $p \leq 0.05$. In addition, interclass correlation described by Lightfoot et al. (2004) was used to estimate broad-sense heritability of cell proliferation in the RMS.

Genome-wide interval mapping of interstrain differences in RMS proliferation was performed using WebQTL, a module of GeneNetwork (http://www.genenetwork.org/webqtl/main.py), which is an online database that contains genotype information on more than 360 mouse strains including the 9 inbred strains being investigated in this study. The likelihood ratio statistic (LRS) was computed to assess genotype–phenotype associations and identify

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