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Heparan sulfate niche for cell proliferation in the adult brain

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ABSTRACT

In adulthood, new neurons and glial cells are generated from stem cells in restricted zones of the brain, namely the olfactory bulb (OB), rostral migratory stream (RMS), subventricular zone (SVZ) of the lateral ventricle, sub-callosum zone (SCZ) and sub-granular layer (SGL) of the dentate gyrus. What makes these zones germinal? We previously reported that N-sulfated heparan sulfates (N-sulfated HS) present in specialized extracellular matrix structures (fractones) and vascular basement membranes bind the neurogenic factor FGF-2 (fibroblast growth factor-2) next to stem cells in the anterior SVZ of the lateral ventricle, the most neurogenic zone in adulthood. To determine to which extent cell proliferation is associated with N-sulfated HS, we mapped N-sulfated HS and proliferating cells by immunohistochemistry throughout the adult mouse brain. We found that cell proliferation is associated with N-sulfated HS in the OB, RMS, the whole germinal SVZ, and the SCZ. Cell proliferation was weakly associated with N-sulfated HS in the SGL, but the SGL was directly connected to a sub-cortical N-sulfated HS+ extension of the meninges. The NS-sulfated HS+ structures were blood vessels in the OB, RMS and SCZ, and primarily fractones in the SVZ. N-sulfated HS+ fractones, blood vessels and meninges formed a continuum that coursed along the OB, SVZ, RMS, SCZ and SGL, challenging the view that these structures are independent germinal entities. These results support the possibility that a single anatomical system might be globally responsible for mitogenesis and ultimately the production of new neurons and glial cells in the adult brain.

The production of new neurons and glia persists throughout adulthood in restricted brain locations termed niches. The niche cells comprise stem cells, progenitor cells, ependymocytes [9] and macrophages [17]. The stem and progenitor cells respond to numerous growth factors and cytokines in a regulating environment that comprise locally produced cell adhesion molecules [4,14,21,22] and extracellular matrix (ECM) molecules [11,19]. The growth factors, which promote stem and progenitor cell proliferation and differentiation, circulate through the brain via the cerebrospinal fluid and eventually reach the neurogenic niche [12]. In the other hand, the ECM and cell adhesion molecules are fixed at the cell surface or in basement membranes, potentially forming the specific niche structures that can capture and promote growth factors to ultimately coordinate stem cell proliferation and differentiation [6,10–12,14,19]. To date, the physiological interactions between ECM, cell adhesion molecules and growth factors in the stem cell niches have not been elucidated. What are the specific ECM or cell adhesion molecules in the neural stem cell niches? Previous publications report that adult neurogenic zones are associated with the polysialylated form of neural cell adhesion molecule (PSA-NCAM) [1,4,5,21,22,24]. PSA-NCAM has been found in the olfactory bulb (OB), subventricular zone (SVZ) of the lateral ventricle, rostral migratory stream (RMS) and sub-granular layer (SGL) of the hippocampus, all neurogenic/gliogenic zones [8,9,13,23] and in sub-pial locations. We have previously shown that proliferating cells in the SVZ of the lateral ventricle are associated with N-sulfated heparan sulfate (N-sulfated HS) proteoglycans [12]. HSPG are highly heterogenous ECM molecules composed of sulfated HS chains attached to a core protein, such as perlecan [2,3]. Numerous growth factors and cytokines, including those promoting stem cell proliferation and differentiation, are heparin-binding molecules [19]. This implies that the growth factor binding to the HS chains of HSPG in the extracellular space is required for growth factor activity [3,6,10,19,20,25]. For, example, fibroblast growth factor-2 (FGF-2) must bind perlecan to promote mitosis in multiple organs and tissues [3,6,25]. It is believed that the pattern of sulfation (N-, O-6, O-3- or O-2-linked) in the glycosylated chains of HSPG is determinant for the selective recognition of growth factors/cytokines [11,19]. We found that N-sulfated HS of the SVZ germinal niche

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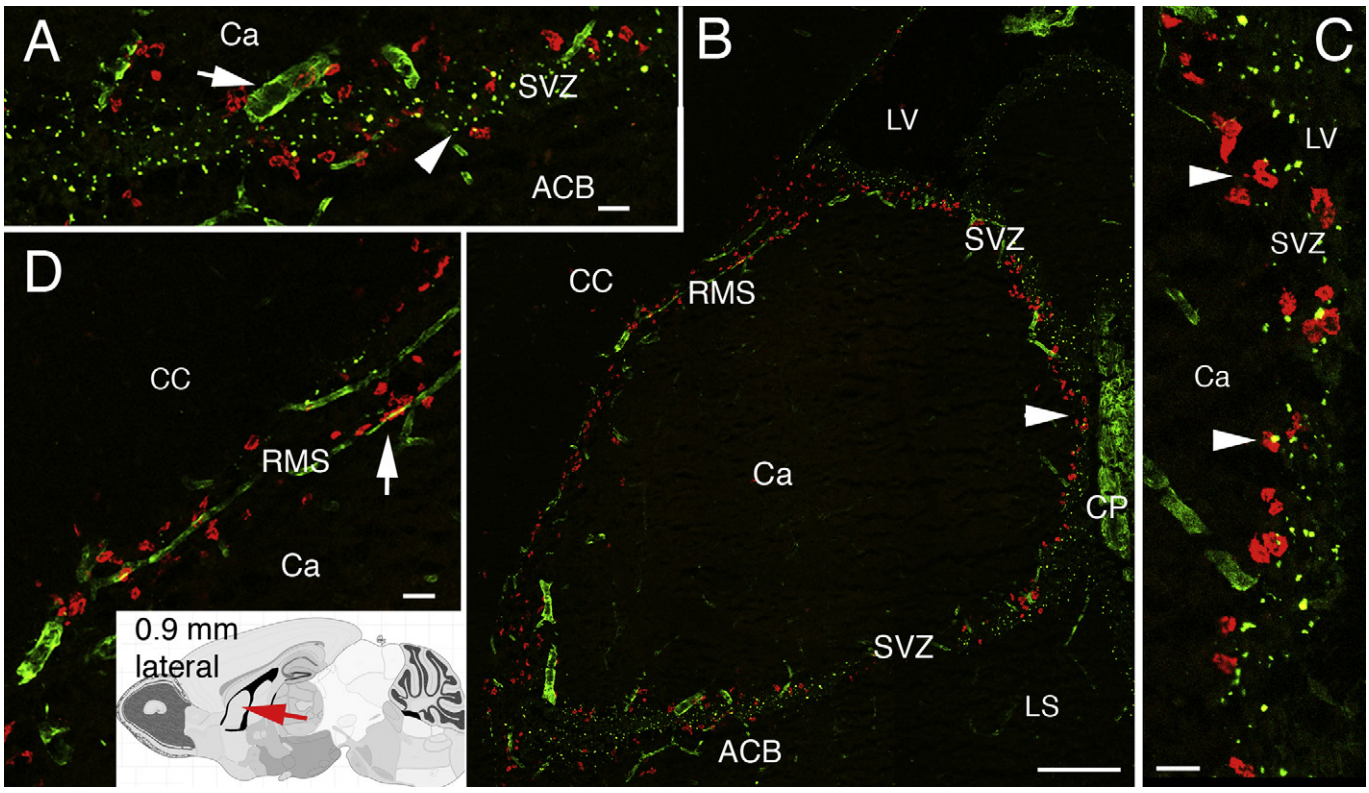

Fig. 1. Cell proliferation is associated with N-sulfated HS in vascular walls, fractone and hybrid vascular/fractone niches. (A) Dual immunolabeling for BrdU (red) and N-sulfated-HS (green) showing cell proliferation next to N-sulfated HS+ fractones (green puncta, arrowhead) and N-sulfated HS+ blood vessels (arrow) in the subventricular zone (SVZ) of a collapsed portion the lateral ventricle at the nucleus accumbens (ACB) surface. Ca: caudate nucleus. The location of this field is indicated by ACB in image (B). (B) Overview of the Ca surface showing the rostral migratory stream (RMS) junction with the SVZ. Proliferating cells are systematically associated with NS-HS immunoreactivity. CP: choroid plexus; LS: lateral septal nucleus. (C) Area of the SVZ (indicated by an arrowhead in (B)) showing cell proliferation associated with N-sulfated HS+ fractones (arrowheads). (D) Magnification of the RMS showing cell proliferation associated with N-sulfated HS+ blood vessels (arrow). The location of all images is shown in the inset. Scale bars. 25 μm in (A, C and D); 200 μm in (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were located in the specialized ECM of the SVZ (fractones, [17,18]) and in the walls of SVZ blood vessels. In addition, N-sulfated HS were responsible for the binding of FGF-2 [12].

In the current study, we sought to determine whether N-sulfated HS are expressed in other germinal zones of the adult brain. We used immunofluorescence histochemistry (IHC) to map proliferating cells and N-sulfated HS throughout the brain in serial sections in the adult mouse.

10–20 week old male and female C57/balb C inbred mice ($n = 10$) were used in this study. The animal experimental protocol followed NIH guidelines and was approved by the Institutional Animal Care and Use Committee at the University of Hawaii. The dissected brains were frozen in isopentane at −80 °C, and series of 25-μm-thick coronal and sagittal sections generated with a Leica CM1900 cryostat (Leica Microsystems Buffalo Grove, IL). Individual sections were identified according to bregma or lateral to midline coordinates. Analysis of the distribution of proliferating cells was assessed by IHC for the thymidine analog boromoeoxyuridine (BrdU) on mice that were terminated 5 h after BrdU intraperitoneal injection (50 μg/kg of body weight). Under these experimental conditions, most immunolabeled cells are in S phase. Therefore, immunolabeling for BrdU reflects the distribution of mitogenesis (mitosis initiation). Bisbenzidine "Hoechst 33258" (2 μg/ml, Sigma–Aldrich, Saint Louis, MO) was used to stain cell nuclei. Dual or triple IHC for BrdU (1/500, OBT 0030, Serotec) and the ECM molecules laminin (L9393, 1/1,000, Sigma–Aldrich) and N-sulfated HSPG (antibody 10E4, 1/500, Seikagaku, Japan) was performed as previously described [12]. These primary antibodies were visualized with Alexa-Fluor 546 goat anti-rat, Alexa-Fluor 647 donkey anti-rabbit, and Alexa-Fluor 488 goat anti-mouse IgM (Molecular Probes/Invitrogen, Carlsbad, CA) respectively. Laminin IHC served as a landmark for basement membranes [12,15–18] in the meninges, choroid plexus, blood vessels and fractones (Figs. 2 and 4) [17,18], which appear as puncta (1–6 μm in diameter) along the ventricle walls (Fig. 2C and D). The antibody 10E4, which is specifically directed against N-sulfate glycosamines [7], labeled fractones and meninges but not the majority of blood vessels (Fig. 2); see also [12]. Perlecan (mAb 1948; clone A7 L6; Millipore, Billerica, MA) visualized with AlexaFluor 546 goat anti-mouse was used as an alternative to laminin to visualize blood vessels, fractones and meningeal basement membranes [12]. The images were recorded with 10× and 20× PlanApo dry objectives using a Leica DFC350FX digital camera mounted on a DMIL epifluorescence microscope (Leica Microsystems, Bannockburn, USA), or a Zeiss Confocal LSM510 microscope. The images were processed and mounted as *X*–*Y* montages (for Figs. 1B, 2B, 3A, 4B, D and E) with Adobe Photoshop CS3 (Adobe Systems, Mountain View, CA). Adjustments for brightness and contrast were minimal.

Fig. 1 shows immunolabeling for proliferating cells (BrdU) and N-sulfated HS at the SVZ/RMS junction. At this location (0.9 mm from the midline), the head of the caudate nucleus fills the cavity of the anterior lateral ventricle, creating two collapsed recesses forming the SVZ/RMS junction (Fig. 1B and schema in D). Each recess consists of two facing ventricle walls containing fractones (green puncta immunoreactive for N-sulfated HS), blood vessels (green tubes) and proliferating cells (BrdU, red) (Fig. 1). The SVZ overlying the caudate is a fractones niche (Fig. 1B and C, arrowheads). The RMS, which does not display fractones, is a vascular niche for