

## Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone

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Received 27 September 2012; revision accepted 7 December 2012

### Abstract

**Objectives:** Fractones are extracellular matrix structures that form a niche for neural stem cells and their immediate progeny in the subventricular zone of the lateral ventricle (SVZa), the primary neurogenic zone in the adult brain. We have previously shown that heparan sulphates (HS) associated with fractones bind fibroblast growth factor-2 (FGF-2), a powerful mitotic growth factor in the SVZa. Here, our objective was to determine whether the binding of FGF-2 to fractone-HS is implicated in the mechanism leading to cell proliferation in the SVZa.

**Materials and methods:** Heparitinase-1 was intracerebroventricularly injected with FGF-2 to N-desulfate HS proteoglycans and determine whether the loss of HS and of FGF-2 binding to fractones modifies FGF-2 effect on cell proliferation. We also examined in vivo the binding of Alexa-Fluor-FGF-2 in relationship with the location of HS immunoreactivity in the SVZa.

**Results:** Heparitinase-1 drastically reduced the stimulatory effect of FGF-2 on cell proliferation in the SVZa. Alexa-Fluor-FGF-2 binding was strictly co-localized with HS immunoreactivity in fractones and adjacent vascular basement membranes in the SVZa.

**Conclusions:** Our results demonstrate that FGF-2 requires HS to stimulate cell proliferation in the SVZa and suggest that HS associated with frac-

tones and vascular basement membranes are responsible for activating FGF-2. Therefore, fractones and vascular basement membranes may function as a HS niche to drive cell proliferation in the adult neurogenic zone.

### Introduction

Fibroblast growth factors (FGFs) compose a family of 24 growth factors that are key players in processes of cell proliferation and differentiation, in a wide variety of tissues and organs, during development and adulthood (1–10). In the adult and developing brain, FGF-2 is a stimulator of neural stem and progenitor cell proliferation and differentiation (3,5,8,9). Although not demonstrated in the adult brain, FGF-2 is known as a heparin-binding protein. This implies that FGF-2 requires interactions with heparan sulphate proteoglycans (HSPG) in the extracellular matrix (ECM) near the cell surface of target cells to recognize and activate FGF receptors and ultimately exert its biological activity (1–7). In addition, binding of growth factors to HSPG is known to protect the growth factors from enzymatic degradation, and provides a mechanism for growth factor storage in the ECM (11). The question arises as to how FGF-2 and other growth factors, with similar or antagonist effects, regulate neural stem and progenitor cell proliferation in the neurogenic niches. Does HSPG promote FGF-2 and other growth factors in the adult neurogenic zones? We have recently demonstrated that N-sulphated HSPGs are highly expressed along the neurogenic axis of the adult brain (12). We found that an N-sulphated HSPG-immunoreactive continuum forms a niche (favourable environment) for cell proliferation, through the germinal zones of the adult brain. These germinal zones comprise the subventricular zone (SVZ) of the lateral ventricle, primary site for adult neurogenesis (13,14), the rostral migratory stream, the olfactory bulbs and the sub-callo-

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sum zone, which all have also been recognized as specific sites for production of new neurons and glia in adulthood (15,16). The N-sulphated HSPG niche consists of ECM structure fractones, which we have previously characterized in the SVZ (17,18), and of vascular basement membranes (12,19). Fractones are small fragments of dense ECM material that are directly visible (without labelling) by transmission electron microscopy (17,18). Along the SVZ of the lateral ventricle, multiple processes of neural stem cells and neural progenitor cells, converge toward each individual fractone (17). Fractones contain HSPG, collagen-IV, nidogen and laminin (17,20) and thus chemically resemble basement membranes (21). However, fractones have punctate morphology when observed by light microscopy and immunolabelling for its ECM components (12,17,18,20), but exhibit fractal (branched) morphology when resolved using transmission electron microscopy (17,18).

Based on our previous findings that biotinylated-FGF-2 *in vitro* binds N-sulphated HSPG, present in fractones and blood vessels of the lateral ventricle walls (20), we hypothesize here that FGF-2 functions as a heparin-binding molecule for regulation of cell proliferation, in this major neural stem cell niche, and that structures responsible for heparin-binding dependence are fractones and nearby vascular basement membranes. To determine whether stimulation of cell proliferation by FGF-2 in the neural stem cell niche is extracellularly mediated by HSPG, here we compare effects of FGF-2 alone with those of FGF-2, after desulphation *in vivo* by heparitinase-1 on cell proliferation in the SVZ of the lateral ventricle. As controls for effects of heparitinase-1 *in vivo*, we examine outcome on cell proliferation, of injecting heparitinase-1 only on disappearance of N-sulphated HSPG immunoreactivity (N-sulphated HSPG+) after heparitinase-1 injection *in vivo*, and potential recovery of N-sulphated HSPG+ after longer post-injection periods. To further examine binding properties of FGF-2, we also compare location of binding of biotinylate-FGF-2 and Alexa-Fluor488-FGF-2, *in vivo* with N-sulphated HSPG+.

## Materials and methods

### *Animals and schedules of intracerebroventricular injection*

Eight to 12-week old male and female Balb/c mice ( $n = 42$ ) were anesthetized with Ketamine/Xylazine mix (ratio 80/20) at 50 mg/kg of body weight. Two successive intracerebroventricular (ICV) injections (lasting 15 min each) were performed unilaterally at bregma +0.5 mm, +0.6 mm lateral to the midline, 2 mm depth

with 1  $\mu$ l of heparitinase-1 (32 mU/injection, from Flavobacterium heparinum, Amsbio, Abingdon, UK), and/or 1  $\mu$ l of FGF-2 (0.5  $\mu$ g per injection, human recombinant lo-FGF-2 17.3 kD produced by *E. coli*, In-Vitrogen, Carlsbad, CA, USA) diluted in artificial cerebrospinal fluid (CSF; Harvard Apparatus, Holliston, MA, USA) using micro-4 microsyringe controller (WPI, Sarasota, FL, USA) connected to stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). ICV injections were performed sequentially on days 1 and 3 with either CSF + CSF (termed CSF condition) or CSF + FGF-2 (FGF-2 condition) or heparitinase-1 + FGF-2 (Hep + FGF-2 condition) or heparitinase-1 + CSF (Hep condition). A single intraperitoneal injection of bromodeoxyuridine (BrdU, 50 mg/kg of body weight; Sigma, St. Louis, MO, USA) was performed 6 h prior to animal termination, on day 5, to assess cell proliferation (pre-mitotic cells). Brains were dissected and frozen in isopentane at  $-80^{\circ}\text{C}$  and stored at  $-20^{\circ}\text{C}$  without fixation. Animal experimental protocols followed NIH guidelines and was approved by the IACUC at the University of Hawaii.

### *In vivo binding of FGF-2*

FGF-2 was either biotinylated using EZ-link Micro-sulfo-NHS Biotinylation kit (21425; Pierce, Rockford, IL, USA) or directly linked to AlexaFluor488 (A30006; In-Vitrogen). After ICV injection, the binding of biotinylated-FGF-2 was revealed on frozen sections of brains, with streptavidin-Texas red. When ICV injections of biotin/AlexaFluor488 only, or artificial CSF only, were carried out, no streptavidin-Texas red or AlexaFluor488 signal was detected in the brains (data not shown).

### *Immunohistochemistry*

Immunohistochemistry (IHC) was performed as previously described, on serial frozen sections generated using a Leica CM1900 cryostat (Leica Microsystems, Buffalo Grove, IL, USA), and was fixed in acetone at  $-20^{\circ}\text{C}$  just prior to IHC procedure (22). Fractones, blood vessels and choroid plexi were immunolabelled with anti-laminin antibodies (1/1000, L9393; Sigma) (13,15) or anti-N-sulphated glycosamine (10E4 antibody, 1/500; Seikagaku) (20,23), then visualized with AlexaFluor-647 goat anti-rabbit antibody (1/400), AlexaFluor-488 goat anti-mouse IgM (1/400, A21042; In-Vitrogen) or Cy-3 donkey anti-mouse IgM (1/400, 715-165-140; Jackson Laboratories, Bar Harbor, ME, USA). For isotype control of 10E4, we immunolabelled frozen sections containing SVZa with IgM anti-chondroitin sulphate CS56 (Abcam, Cambridge, MA, USA) (IgM). Visualization of chondroitin sulphates with CS56

yielded labelling very distinct from 10E4 labelling in the SVZa (Fig. 3e). Pre-mitotic cells (S-phase) were labelled with anti-BrdU antibodies (1/500, OBT0030; Oxford Biotechnology, UK) as previously described (16,20) and visualized with AlexaFluor-488 goat anti-rat antibody (1/400, A11006; InVitrogen) or AlexaFluor-546 goat anti-rat (1/400, A11081; InVitrogen). Results were recorded using a Zeiss confocal laser scanning microscope and images were processed using Adobe Photoshop CS3 (Adobe Systems, Mountain view, CA, USA). Adjustments for brightness and contrast were minimal. Control experiments for immunolabelling specificity were performed as previously described (17,20,22).

#### Cell counting and statistical methods

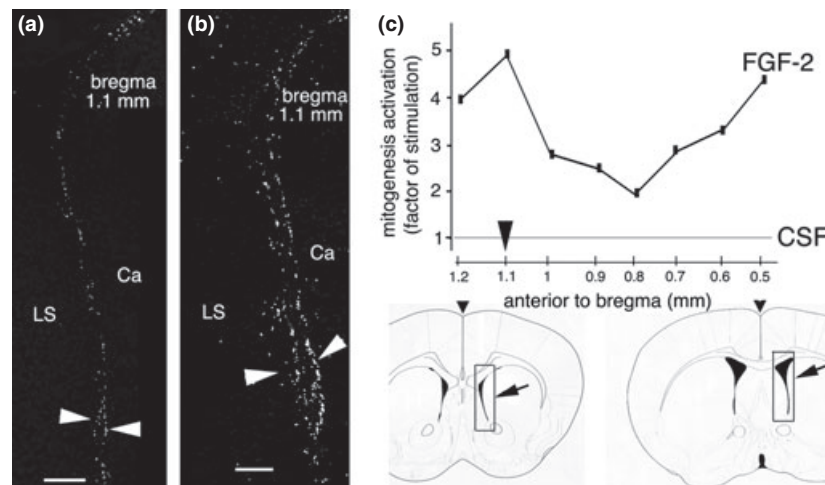
BrdU+ cells were counted in the anterior SVZ (SVZa) of the lateral ventricle, a major proliferation zone in the adult brain, in series of whole-brain 25- $\mu$ m thick coronal sections, using a 20 $\times$  Plan Apo objective and a DMIL Leica immunofluorescence microscope. BrdU+ cell counts were reported per lateral ventricle wall by increments of 0.1 mm, from 1.2 to 0.5 mm anterior to bregma. To compensate for over-counting, observed numbers of BrdU+ cells were subjected to corrections given by Abercrombie's formula (24). Corrected numbers of BrdU+ cells were submitted to statistical analysis

with XLStat. Means and respective standard deviations were reported *per* SVZa for each condition. One-way ANOVA ( $\alpha = 0.01$ ), Tukey's and two-sided Dunnett's tests were employed to compare each condition to the control (CSF condition) and REWQ's procedure for multiple comparison tests were performed to evaluate significance of differences observed. Confidence interval of 99% was applied and a *P*-value inferior to 0.001 was obtained for all comparisons except when comparing CSF and Hep conditions ( $P > 0.001$ ). All statistical models applied had significant difference between 2 conditions.

## Results

#### Upregulation of cell proliferation by FGF-2 along the rostro-caudal axis of the anterior SVZ

We first examined effects of FGF-2 on cell proliferation along the rostro-caudal axis of the anterior SVZ (SVZa). Figure 1a shows typical cell proliferation at 1.1 mm anterior to bregma (bregma +1.1 mm) after CSF injection (control). At this location, the lateral ventricle is collapsed (arrows). At bregma +1.1, mitogenesis was higher by a factor 5 in the SVZa, after injection of FGF-2 (Fig. 1b arrowheads, 1c) in comparison to controls, that is injection with artificial CSF. Along the rostro-caudal axis, the elevation was between 2 and 5-fold



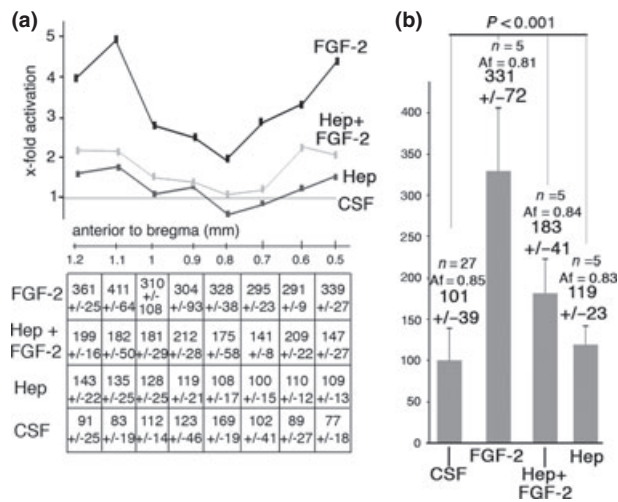
**Figure 1.** Upregulation of cell proliferation by FGF-2 along the rostro-caudal axis of the SVZa of the lateral ventricle (LV). (a) Cell proliferation was assessed by BrdU immunoreactivity in the SVZa of the LV after ICV injection of CSF (control condition). Proliferating cells (BrdU+ puncta) are located along collapsed walls of the LV (arrowhead). Ca: caudate nucleus; LS: lateral septal nucleus. (b) Increased cell proliferation after ICV injection of FGF-2. Numerous BrdU+ puncta are found along the walls of the LV (arrowhead). (c) Graph showing upregulation of cell proliferation along the rostro-caudal axis of the SVZa. Numbers in vertical axis indicate factors by which cell proliferation is increased after FGF-2 injection (compared to cell proliferation after CSF injection, control). Data are reported at different locations along the SVZa, from +1.2 to +0.5 mm anterior to the bregma line. Schematic representations of the brain indicate locations where BrdU+ cells were counted (frames shown by arrows). Images (a) and (b) correspond to frames at +1.1 mm anterior to bregma. Scale bars: 100  $\mu$ m.



(Fig. 1c) with average factor of 3.3 when merging results from bregma +1.2 to +0.5 mm (Fig. 2b).

*Upregulation of cell proliferation by FGF-2 requires N-sulphated HSPG*

To determine whether the mitogenic effect of FGF-2 was heparan sulphate-dependant, we compared effects of FGF-2 on cell proliferation in the SVZa after successive injections of CSF and FGF-2, and of heparitinase-1 and FGF-2 [heparitinase-1 cuts N-sulphated chains of HSPG (25,26)]. In control experiments, we examined effects of injection of heparitinase-1 only on cell proliferation in the SVZa. Heparitinase-1 alone did not markedly increase the level of cell proliferation in the SVZa, compared to injection of CSF alone (Fig. 2b). When heparitinase-1 was injected prior to FGF-2, the mitogenic effect of FGF-2 was drastically lower throughout the SVZa (Fig. 2a). Merging data from +1.2 to +0.5 mm to bregma, the level of cell proliferation in the SVZa increased by a factor 1.7 after heparitinase-1+ FGF-2, compared to a factor of 3.3 when FGF-2 only was injected (Fig. 2b). These results demonstrate that heparan sulphates promote the mitogenic effect of FGF-2 in the SVZa.



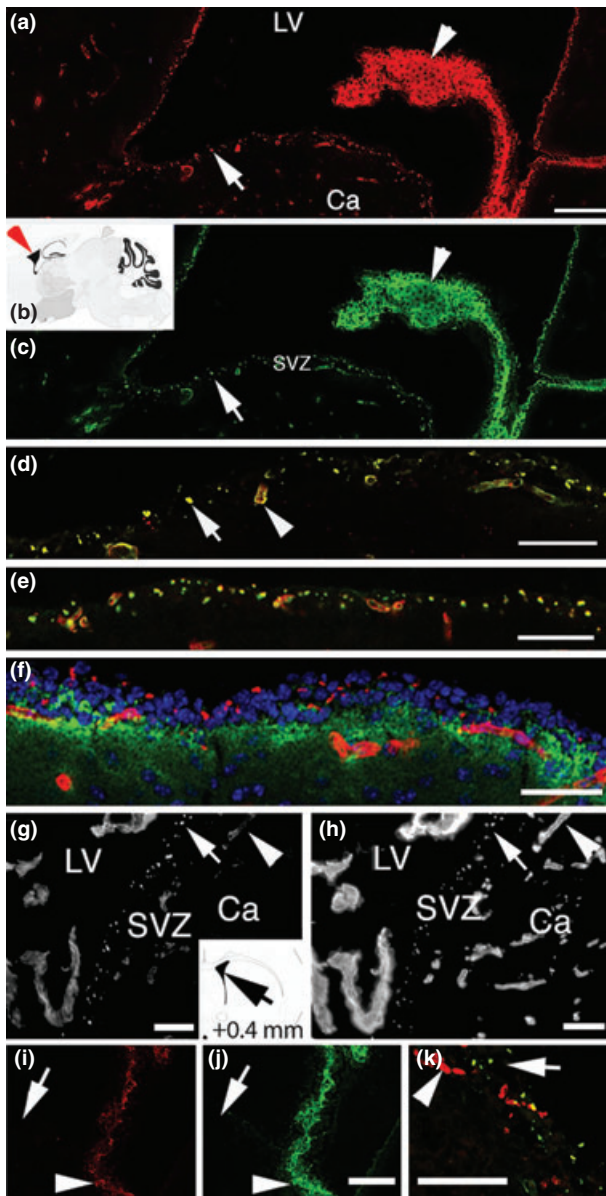
**Figure 2. Upregulation of cell proliferation by FGF-2 requires heparan sulphates.** (a) Co-injection of heparitinase-1 and FGF-2 (Hep + FGF-2) strongly inhibited stimulatory effects of FGF-2 on cell proliferation in the SVZa. Graph indicates the factor by which cell proliferation was increased along the rostro-caudal axis of the SVZa. Results obtained after injection of heparitinase-1 only, are also reported in the graph. All factors were normalized to values (number of BrdU+ cells) obtained after injection of CSF (control). Bottom panel shows mean values and standard deviations for all sections from 1.2 to 0.5 mm anterior to the bregma. (b) Graph showing BrdU+ cell average values for the entire zone from +1.2 to +0.5 mm anterior to the bregma. FGF-2 upregulated cell proliferation by a factor 3.1. Co-injection of heparitinase-1 reduced the effect of FGF-2 to a factor 1.8.

*Binding of FGF-2 in vivo co-localizes with N-sulphated HSPG immunoreactivity*

Previously, we have demonstrated that biotinylated-FGF-2 binding *in vitro* in frozen brain sections co-localizes with N-sulphated HSPG, and that binding of biotinylated-FGF-2 *in vivo* coincides with laminin+ puncta or blood vessels, in the SVZa (20). However here, we did not demonstrate any correlation between FGF-2 binding *in vivo* and N-sulphated HSPG immunoreactivity. FGF-2 was biotinylated or directly coupled to AlexaFluor-488, then ICV injected in adult mice. Twenty-two hours after injection, FGF-2 binding sites were observed after incubation of sections with streptavidin-Texas red (Fig. 3a), or were directly observed on the 488 nm channel by fluorescence microscopy (Fig. 3g). To determine whether binding loci of FGF-2 *in vivo* would coincide with those of N-sulphated-HSPG, brain sections originating from animals that were injected with biotinylated FGF-2 were immunolabelled with 10E4 antibody, raised against N-sulphated HSPG. Binding sites of biotinylated-FGF-2 and of AlexaFluor-488-FGF-2 almost perfectly matched patterns of immunoreactivity for N-sulphated HSPG (Fig. 3a–d, 3g and 3h) as indicated by yellow colouration, overlap of 10E4 labelling (green) and FGF-2 binding (red) (Fig. 3d). In the SVZa, most FGF-2/N-sulphated-HSPG+ co-labelling was found in fractones, which typically appeared as small puncta <5 µm in diameter (13,16), although blood vessel walls (which are larger), and as truncated cylindrical structures, were also clearly visualized (Fig. 3d, arrowhead). Structural and ultrastructural differences between fractones and blood vessels has demonstrated in our previous studies (17,18).

FGF-2 binding was not found in the SVZa after heparitinase -1 treatment (dose equal to that used for investigating effects of heparitinase-1 on cell proliferation) (Fig. 3i, arrow). N-sulphated HSPG+ also disappeared from the SVZa after heparitinase-1 injection (Fig. 3j), although N-sulphated HSPG+ remained in the choroid plexus (Fig. 3j, arrowhead). This demonstrates that FGF-2 binding in the SVZa required N-sulphated-HSPG. This also indicates that FGF-2 was unable to bind in the SVZa with experimental conditions used to investigate heparitinase-1 effects on cell proliferation. Thus, our results support the view that when FGF-2 cannot bind fractones and SVZa blood vessels, it cannot stimulate cell proliferation in the SVZa.

To further validate specificity of FGF-2 binding in the SVZa, we injected biotinylated-FGF-2 into the dorsal cortex, instead of into the lateral ventricle. Biotinylated-FGF-2 was captured by N-sulphated-HSPG+ fractones and blood vessels in the SVZa (Fig. 4f), demonstrating that FGF-2 binding sites were independent of injection site in the brain. We also show that



biotinylated FGF-2 was captured by N-sulphated-HSPG+ fractones and blood vessels in the SVZ of the third ventricle after injection into the lateral ventricle (Fig. 4c). Together, these experiments demonstrate that capture of biotinylated-FGF-2 by fractones and vascular basement membranes of the SVZ was independent of injection site and was not restricted to the SVZa.

To investigate toxicity induced by heparitinase-1 treatment, we examined dynamic of recovery of N-sulphated HSPG+ after heparitinase-1 injection. Figure 3f shows that N-sulphated HSPG+ had fully recovered 48 h after heparitinase-1 injection. Thus, our experimental conditions were appropriate for examining effects of heparitinase-1 on cell proliferation in the SVZa.

**Figure 3. FGF-2 binding *in vivo* matched location of fractone-HSPG+.** (a) Binding of biotinylated-FGF-2 visualized by streptavidin-Texas red 22 h after ICV injection. Binding of biotinylated-FGF-2 was concentrated in the SVZa (arrow) and choroid plexus (CP) (arrowhead). Ca: caudate nucleus. (b) Location of images a-f. (c) Immunolabelling for N-sulphated-HSPG (10E4, green) in the same section showing distribution pattern identical to binding of biotinylated-FGF-2. Numerous fractones, visualized as 10E4+ puncta (arrow in b) have concentrated biotinylated-FGF-2 (arrow in a). (d) High power field of the SVZa (indicated by an arrow in a) displaying biotinylated-FGF-2 binding and 10E4+ simultaneously. Yellow colouration indicates that the vast majority of 10E4+ fractones (arrow) and 10E4+ blood vessels, visualized as truncated cylinders (arrowhead), have bound FGF-2. (e) Double immunolabelling for laminin (red) and N-sulphated HSPG (green). (f) Isotype control for 10E4, showing IgM antibody (CS56) directed against chondroitin sulphates (green). The same secondary antibody (Alexa-Fuor 488 goat anti-mouse IgM) was used to reveal chondroitin sulphates (in e) and 10E4 (in b-d). IgM directed against chondroitin sulphates does not recognize fractones and blood vessels, which here are visualized by laminin+ (red). (g) Binding of AlexaFluor-488-FGF-2 visualized 24 h after ICV injection. LV: lateral ventricle. (h) Laminin+ in the same section showing that Alexa-Fluor488-FGF-2 binding matches location of laminin+ in fractones (arrow), CP (double arrowheads) and blood vessels (arrowheads) in the SVZa. Blood vessels located beyond the SVZa do not bind FGF-2 (double arrows). (i) Loss of biotinylated-FGF-2 binding (red) in the SVZa 22 h after injection of heparitinase-1 (arrow). (j) N-sulphated-HS+ (green). Biotinylated FGF-2 binding and N-sulphated HS+ remained in the CP stroma, suggesting lack of access of heparitinase-1 to the CP, or rapid recovery of N-sulphated-HSPG+ and FGF-2 binding in the CP (arrowhead). (k) Recovery of N-sulphated-HSPG+ (green) in the SVZa 48 h after heparitinase-1 injection. Presence of 10E4+ puncta indicates that fractones have recovered heparan sulphates. Red colouration shows typical BrdU+ cell proliferation in the SVZa. Scale bars: 100 µm.

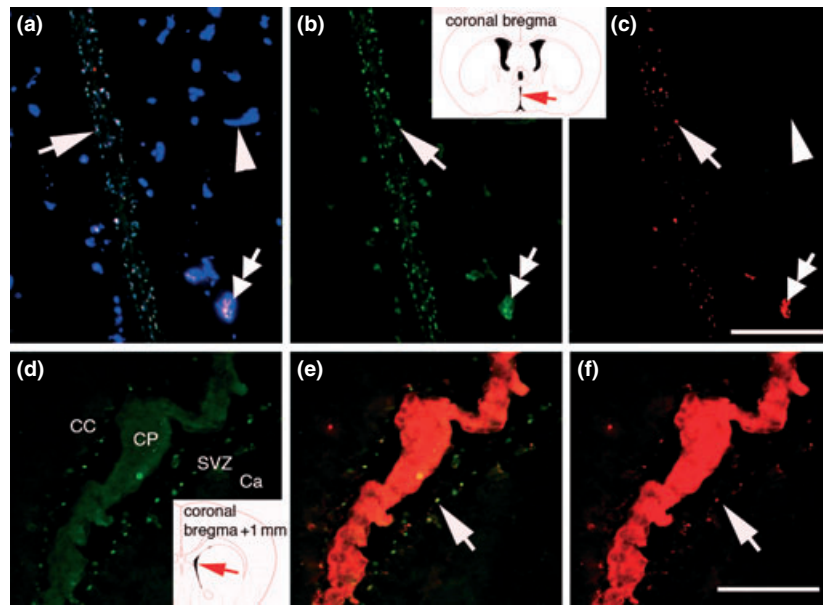
## Discussion

### *FGF-2 required heparan sulphates to stimulate cell proliferation in the SVZa*

In this study, we demonstrated *in vivo* that stimulation of cell proliferation induced by FGF-2 in the SVZa required heparan sulphates. This is the first demonstration that heparan sulphates mediate FGF-2-induced mitosis in the neural stem-cell niche of the adult brain. We have recently shown that inhibition of cell proliferation by BMP-7 in the SVZa neurogenic zone also requires heparan sulphates (27). This supports the view that heparan sulphates play a significant role in regulation of cell proliferation in the adult brain.

### *Loss of FGF-2 effect on cell proliferation was associated with loss of heparan sulphates in fractones and SVZ vascular basement membranes*

We have previously demonstrated that FGF-2 primarily binds to SVZa fractones and blood vessels, and that a mix of heparitinase-/heparitinase-3 prevents this binding (20). Here, we used heparitinase-1 to cut N-sulphated



**Figure 4.** FGF-2 capture by fractones and basement membranes was independent of injection site. (a–c) Binding FGF-2 visualized by streptavidin Texas red in SVZ of the third ventricle, 22 h after ICV injection. FGF-2 binding (red) and immunolabelling for laminin (blue), 10E4 (green) and are all displayed in image (a). Image (b) shows 10E4 only and image (c) shows FGF-2 binding only. Due to construction of the third ventricle, fractones appear as two parallel lines (arrows). Numerous fractones (arrow in b) captured and concentrated biotinylated FGF-2 (arrow in c). Specific blood vessels of the SVZ captured FGF-2 (double arrow), most blood vessels did not (arrowheads). Inset indicates location of images. (d–f) 10E4 immunolabel (green, d) and binding of biotinylated FGF-2 (red, f) displayed together in E, 22 h after injection in the dorsal cortex. Fractones of the lateral ventricle SVZ (arrow in E) wall concentrated FGF-2 (arrow in f). Note that the choroid plexus also concentrated FGF-2. Inset in (d) indicates the location of images. Scale bars: 100  $\mu\text{m}$ .

HSPG specifically. We found that both biotinylated-FGF-2 and AlexaFluor-488-FGF-2 bound *in vivo* to sites that match N-sulphated HSPG+ fractones and blood vessel basement membranes, of the SVZa. Interestingly, most vascular basement membranes (besides those of the SVZa) were neither NH-HS+ nor bound FGF-2. Therefore, location of N-sulphated HSPG may reflect a specific niche for FGF-2 binding and activation. After *in vivo* heparitinase-1 desulphation, both FGF-2 binding to fractones/vascular basement membranes and FGF-2-mediated stimulation of cell proliferation were lost. Together, these results strongly suggest that FGF-2 must bind N-sulphated HSPG located into fractones and the SVZa vasculature, to stimulate cell proliferation.

#### *Heparan sulphates act as captors and activators of growth factors, cytokines and chemokines*

The importance of heparan sulphates as ECM receptors that bind and potentiate growth factors has previously been emphasized. HSPG have been implicated as crucial co-receptors for interaction with a large variety of ligands including growth factors FGF-1, FGF-2 and FGF-4 (1–4,28–30), as well as other members of the FGF family, VEGF (vascular endothelial growth factor

(31), HB-GAM (heparin-binding growth-associated molecule) (32), BMP (bone morphogenic protein) (27,33), SCF (stem cell factor) (34), HGF (hepatocyte growth factor) (35), amphiregulin (36), transforming growth beta-1 (TGF $\beta$ -1) (37), cytokines such as granulocyte-macrophage colony stimulating growth factor (GM-CSF), (38,39) (G-CSF), IL (interleukins) (40) and IFN (interferons) (41), CC chemokines (42) and CXC chemokines (43), and diverse morphogens such as Shh (sonic hedgehog) (44) and Noggin (45). All these heparan sulphate-binding signalling molecules influence neurogenesis in adulthood (46–57). However, with the exception of BMP-7 (our own previous study, 27) heparin-binding dependence of these growth factors has not been reported in the adult brain.

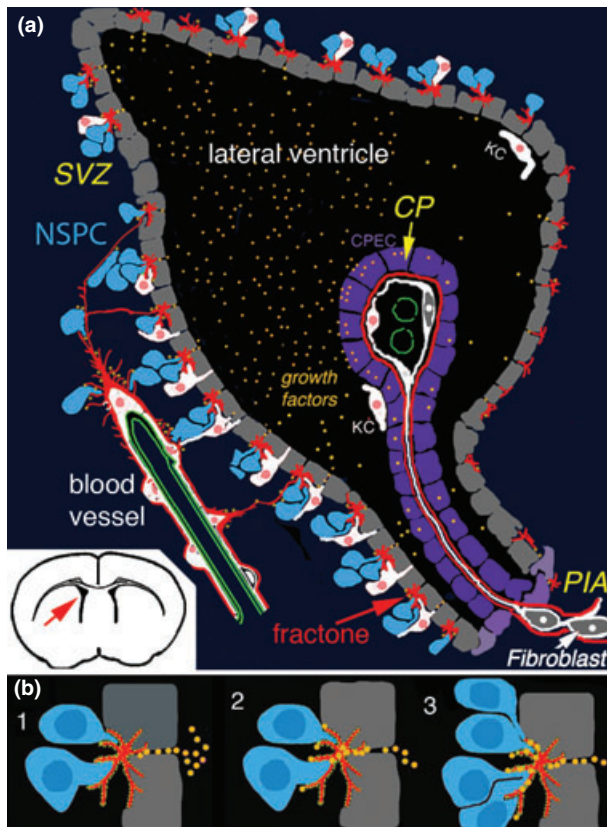
#### *Experimental validation, binding specificity, diffusion and toxicity of FGF-2 and heparitinase-1*

In this study, we have demonstrated that binding of biotinylated-FGF-2 to fractones and vascular basement membranes in the SVZa is independent of site of injection (Fig. 4). FGF-2 is known to slowly diffuse through brain tissue, and has slow clearance and low rate of metabolic degradation (58). Thus, biotinylated-FGF-2 can



diffuse from the cortex to the sub-cortical structures (on top of penetrating from the ventricles) prior to binding fractones and blood vessels in the SVZa. These characteristics explain why biotinylated-FGF-2 bound to fractones and vascular basement membranes of the SVZa 22 h after injection into the cortex (Fig. 4f).

Twenty-two hours after injection into the cortex or lateral ventricle, biotinylated-FGF-2 was also visualized in the stroma (but not significantly in the epithelium) of the choroid plexus (Figs 3a arrowhead, 4f). This was predictable as FGF-2 is capable of retrograde transport from the ventricle to the choroid plexus stroma, despite



**Figure 5. Hypothetical model of growth factor capture and activation in the SVZa neurogenic zone of the adult brain.** (a) Overview of organization of the neurogenic zone (SVZ lateral ventricle). Neural stem cell and progenitor cells (NSPC) directly contact fractones just beneath the ependyma (cell layer facing the ventricle, represented in grey). Blood vessel, which also contacts NSPC, completes the neurogenic niche. Growth factors, such as FGF-2, are secreted by the choroid plexus (CP), enter the interstitial clefts between ependymocytes, are captured by fractones and eventually by blood vessels of the niche. Kolmer cells (KC) and macrophages associated with fractones are represented in white. (b) Mechanism of FGF-2 capture and activation in SVZ neurogenic zone. 1. FGF-2 enters interstitial clefts. 2. FGF-2 is captured by heparan sulphates (small yellow) located at the fractone surface, then is presented to its cognate receptors at the NSPC surface. 3. NSPC that are activated by FGF-2, proliferate.

the CSF barrier formed by the choroid plexus epithelium (59,60). Knowing that fibroblasts are ordinary targets for FGF-2 (61), we anticipated that biotinylated-FGF-2 would bind to fibroblasts in the stroma of the choroid plexus. In contrast to biotinylated-FGF-2, heparitinase-1 was unable to benefit from retrograde transport through the CSF barrier of the choroids plexus and did not prevent binding of biotinylated-FGF-2 to the N-sulphated-HSPG in the choroid plexus stroma (Fig. 3i).

It is important to note that low molecular weight (17.3 kD) FGF-2, also named lo-FGF-2, was used in this study. Lo-FGF-2 is incapable of causing chromatin compacting and cell death (62,63). On the contrary, high molecular weight 23kD FGF-2 (hi-FGF-2) has been shown to cause substantial cell death (63). Finally, we presume that heparitinase-1 had no effect on cell death in our experimental procedures. According to results obtained by literature searching, 10 mU of heparitinase had no effect or reduced cell death (depending on cell layers) on retinal explants in culture (64).

In conclusion, our experiments demonstrate for the first time that N-sulphated HSPGs are essential for stimulation of cell proliferation by FGF-2 in the SVZa. Moreover, location of FGF-2 binding in SVZa fractones and blood vessels, and necessity of this binding to mediate FGF-2 stimulation of cell proliferation, strongly suggest that fractones and SVZa blood vessels specifically capture FGF-2 to control the level of cell proliferation in the adult SVZa. High concentration of N-sulphated HSPG in fractones and vascular basement membranes makes the SVZa unique and particularly suitable for recruiting and dispatching growth factors to neural stem and progenitor cells. We hypothesize a mechanism of FGF-2 activation by fractone-heparan sulphates in the context of the anatomy of the SVZa (Fig. 5). As FGF-2 is produced highly by the choroid plexus epithelium (65), FGF-2 may enter between ependymocytes *via* interstitial clefts (66) to finally reach fractones and neural stem cells in the SVZa. Knowing that numerous heparin-binding growth factors and cytokines are produced by the choroid plexus and released in the ventricles (65,67), we propose that fractones collect growth factors/cytokines from the ventricles and activate these signalling molecules in the ventricle walls (SVZ) *via* heparin-binding controlled mechanisms (68).

## Acknowledgements

This work was supported by NIH R21 NS057675 and JSPS S09109. We thank the Histology and Imaging Core supported by RCMI G12-RR003061 and COBRE P20-RR016453. We thank Chloe Banel for proofreading this article.

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