Cell. Mol. Life Sci. 64 (2007) 1254 – 1258
1420-682X/07/101254-5
DOI 10.1007/s00018-007-6476-5

Cellular and Molecular Life Sciences

Review

Expression and function of galectin-1 in adult neural stem cells

M. Sakaguchi, Y. Imaizumi and H. Okano*

Department of Physiology, Keio University School of Medicine, 35 Shinanomachi, Tokyo 160-8582 (Japan), Fax: +81 3 3357 5445, e-mail: hidokano@sc.itc.keio.ac.jp

Received 6 November 2006; received after revision 13 December 2006; accepted 15 February 2007
Online First 15 March 2007

Abstract. Neural stem cells (NSCs) in the adult mammalian brain proliferate and continuously produce new neurons. To date, there has been little research into the functions of lectins in adult NSCs. Recently, we reported that a lectin, galectin-1, is expressed on adult NSCs and promotes their proliferation through its carbohydrate-binding ability. This evidence raises the possibility that glycans play roles in the proliferation of adult NSCs.

Keywords: Lectin, galectin, neural stem cell, glycan, subventricular zone astrocyte.

Introduction

Neural stem cells (NSCs) proliferate throughout life and differentiate into young neurons in the adult mammalian brain [1–5]. They are the primary progenitor cells of the neurons that turn over in the olfactory bulbs (OB) and in the dentate gyrus (DG) of the hippocampus, the principal regions for olfaction and memory, respectively. Recent studies have shown the expression of glycans on NSCs and in their niche [6,7], suggesting that the glycans have functional significance [8]. To function, glycans interact with carbohydrate-binding proteins (*e.g.*, lectins) in many biological settings. However, the function of lectins on adult NSCs has not been elucidated.

Galectin-1 is a lectin that preferentially binds to the lactosamine structure in glycans [9–11]. Galectin-1 is expressed by various stem cells, including embryonic, hematopoietic, and keratinocyte stem cells [12–16]. However, whether NSCs express galectin-1 *in vivo* and how it might function have been unknown. Here, we review the recent data on the expression and function of galectin-1 in adult NSCs.

NSCs in the adult mammalian brain

NSCs reside in two anatomically distinct regions in the adult mammalian brain: the subventricular zone (SVZ) of the lateral wall of the lateral ventricles (Fig. 1) and the subgranular zone of the DG of the hippocampus. Several types of progenitor cells are produced in a hierarchical manner from NSCs (Fig. 1D). NSCs in the SVZ have certain characteristics of astrocytes, such as GFAP expression [17]. (Hereafter, we refer to these cells as SVZ GFAP-expressing cells, to distinguish them from astrocytes in other regions.) NSCs proliferate and produce transit-amplifying cells (TAs). TAs rapidly proliferate, increasing in number, and differentiate into neuroblasts. The neuroblasts migrate to the OB, where their final differentiation into interneurons takes place. Each progenitor type in the SVZ can be distinguished by its expression pattern of molecular markers (Fig. 1E) [3, 18]. The NSCs of the DG and their functional significance for adult neurogenesis are thoroughly discussed in other reviews [2, 3].

* Corresponding author.

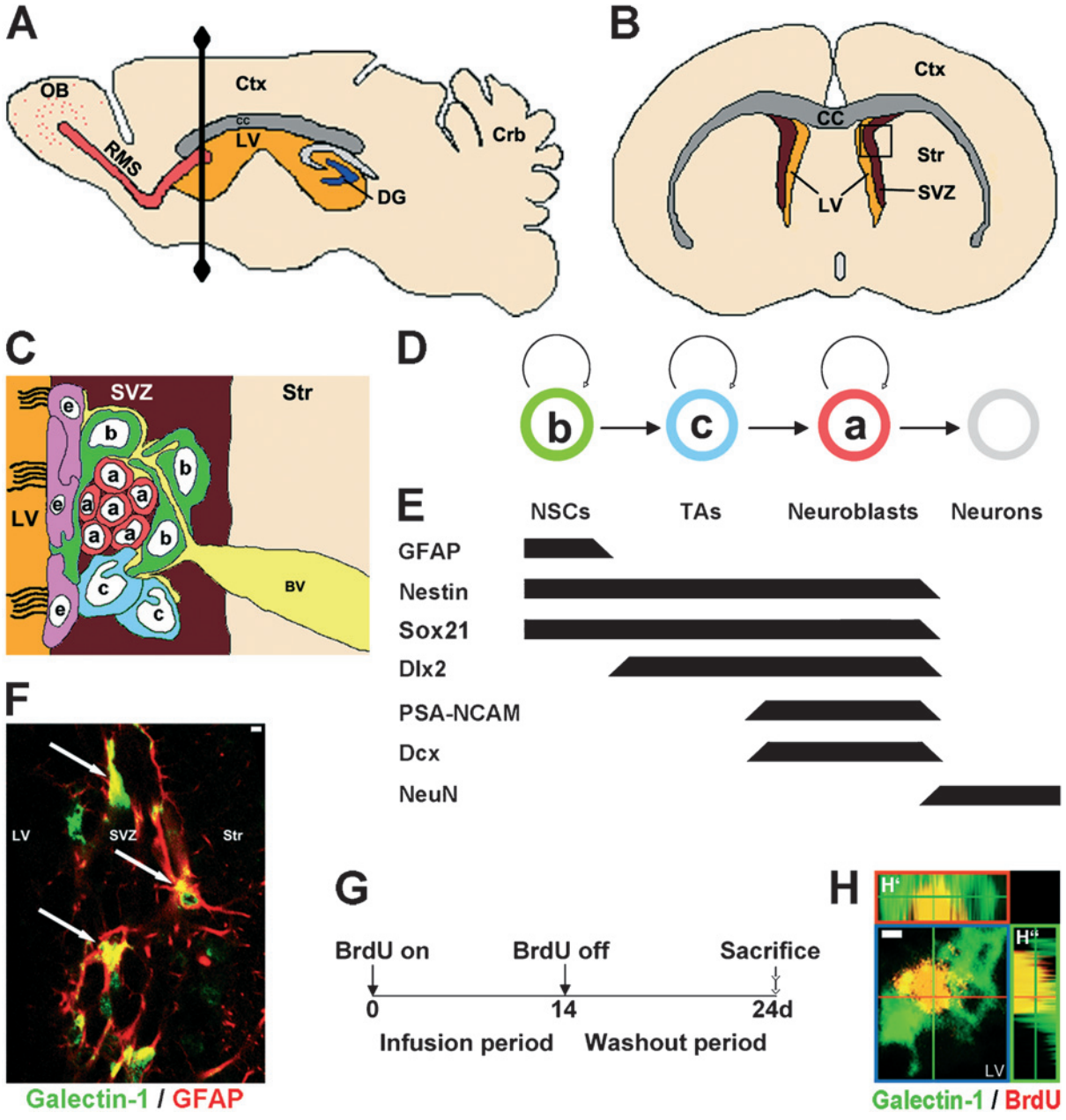

Figure 1. Galectin-1 is expressed in the neural stem cells (NSCs). (*A*) Illustration of a sagittal section of adult mouse brain showing the extent of the lateral ventricle (orange) where the subventricular zone (SVZ) exists. (*B*) Illustration of a coronal brain section taken at the rostral-caudal position shown as a black line in (*A*). The SVZ (brown) is shown as a thin layer between the lateral ventricle and striatum. In practice, it is easily identified by its cell density, which is higher than that of the striatum. (*C*) Drawing of a magnified view of the part of the brain shown boxed in (*B*). A single layer of ependymal cells (*E*) lies between the lateral ventricle and the SVZ. This layer is believed to be permeable, allowing various molecules to enter the SVZ [41]. (*D*) Hierarchy of adult neurogenesis in the SVZ. NSCs (*B*) slowly divide to generate transit-amplifying cells (TAs) (*C*), which proliferate rapidly and produce neuroblasts (*A*). Neuroblasts migrate through the rostral migratory stream [RMS: shown as a red line in (*A*)] into the olfactory bulbs (OB) where they differentiate into neurons. (*E*) Marker expression pattern for each stage of neural cell differentiation in adult neurogenesis. (*F*) A subset of SVZ cells, indicated by white arrows, co-express galectin-1 (green) and GFAP (red). Slice thickness, 1 μm. (*G*) Time schedule for long-term BrdU labeling. BrdU was infused for 14 days, and the mice were killed 10 days later. (*H*) A long-term BrdU-retaining cell, which is positive for galectin-1. H′,H″: 3-D reconstruction images. Slice thickness, 1 μm. Ctx, cortex; CC, corpus callosum; LV, lateral ventricle; DG, dentate gyrus; Crb, cerebellum; Str, striatum; BV, blood vessel. Scale bars in (*F, H*): 4 μm. Figures are modified from [8].

Table 1. The galectin-1 expression and functions in the nervous system as reported in the literature (Ox, the functions of oxidized galectin-1; R, the functions of reduced galectin-1; DRG, dorsal root ganglion)

Locus	Subtype	Embryo	Postnatal	Adult	Ref.	Function	Ref.
Spinal cord	Sensory neurons in DRG	+	+	+	[19–23]		
(Brain stem)	Motor neurons	+	+	+	[19–23]	Neurite growth in injury (Ox)	[30, 37]
	Schwan cells			+	[24, 25]	Migration after injury (Ox)	[24, 30]
	Red nucleus complex			+	[22]		
Cerebellum	Neurons		+	+	[26]		
	Glial cells		+	+	[26]	Differentiation (R)	[32]
Forebrain	Neurons		+	+	[27]		
	SVZ astrocytes			+	[18]	Proliferation (R)	[18]
Olfactory bulb	Sensory neurons	+	+	+	[28]	Neurite growth and/or pathfinding (R)	[31]
	Mitral cells		+	+	[28]		
	2nd order projection neurons			+	[28]		
	Interneurons			+	[28]		
	Non neuronal cells			+	[29]		

Galectin-1 expression in the adult CNS

Although several reports have shown the expression of galectin-1 in the CNS (Table 1) [19–32], its expression in NSCs had not been shown until recently [18]. In the adult mouse SVZ, we found galectin-1 expression in the GFAP-expressing cells (Fig. 1F), which were also positive for Nestin [18], a marker of neural progenitor cells in the adult brain. To confirm the expression of galectin-1 in adult NSCs, we used the BrdU long-labeling method (Fig. 1 G). BrdU is a thymidine analog that is taken up by dividing cells in S phase. It is known that adult NSCs divide more slowly than other progenitors in the SVZ [33]; therefore, once they take up BrdU, they can retain it for an extended time (the wash-out period). In the wash-out period, TAs proliferate and dilute the incorporated BrdU, and neuroblasts migrate out of the SVZ. Thus, after the wash-out period, the BrdU-labeled cells (long-term BrdU-retaining cells) in the SVZ are highly enriched for NSCs [17]. We confirmed the expression of galectin-1 in a subset of the long-term BrdU-retaining cells (Fig. 1 H) [18]. These results indicate that galectin-1 is expressed in adult NSCs.

Galectin-1 promotes the proliferation of adult NSCs

The neurosphere assay (Fig. 2A) is a method to study the characteristics of neural progenitor cells *in vitro* [34, 35]. In the neurosphere assay, dissociated tissue containing neural progenitor cells is cultured in serum-free medium supplemented with defined growth factors. The medium formulation allows neural progenitors, which could include NSCs and TAs [36], to proliferate and form spherical cell aggregates, called neurospheres. Cells in the neurospheres can differentiate into neurons and glial cells. This makes it possible to study the two basic characteristics of neural progenitor cells (*e.g.*, proliferation and differentiation) *in vitro*.

To study the function of galectin-1 in adult neural progenitor cells *in vitro*, we infused recombinant galectin-1 (rGalectin-1) into the lateral ventricle, so that rGalectin-1 could reach the SVZ (Fig. 2A). The SVZ was subsequently dissected and cultured to form neurospheres. We found that the number of neurospheres formed from the SVZ of the rGalectin-1-infused brain was increased compared with that of the saline-infused control brain (Fig. 2B). The percentages of neurons that differentiated from the neurospheres of the rGalectin-1- or saline-infused brains were not significantly different [18]. These results suggest that the rGalectin-1 infusion increased the number of NSCs and TAs in the SVZ.

To confirm this result *in vivo*, we infused BrdU along with rGalectin-1, and the number of long-term BrdU-retaining cells was counted. As expected, the number was significantly higher in the rGalectin-1-infused brains than in the saline-infused control brains [18]. We also studied the markers expressed in the SVZ cells after infusion. We confirmed that the cell population that fulfills the criteria for NSCs and TAs was significantly increased after the rGalectin-1 infusion (Fig. 2C) [18]. In addition, we found no significant difference in the number of apoptotic cells after the infusion [18]. Moreover, *galectin-1*-knockout mice showed a reduction in the number of NSCs (Fig. 2D) and TAs [18]. Together, these results

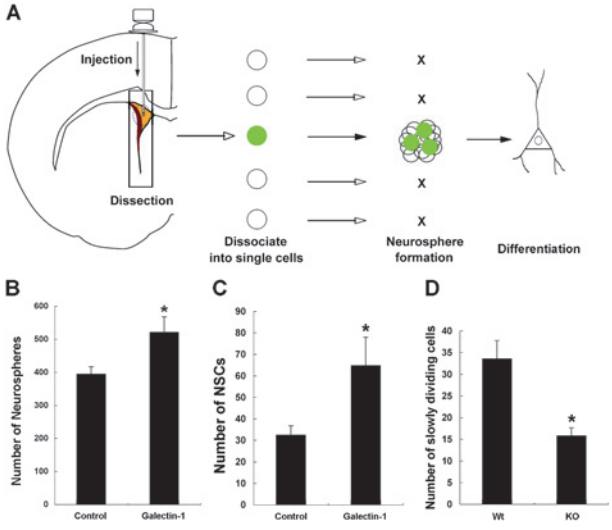

Figure 2. Galectin-1 promotes proliferation of adult NSCs. (*A*) Schematic illustration of the experimental procedure. rGalectin-1 was infused by osmotic pump into the lateral ventricle (orange). Tissue (boxed) that included the SVZ (brown) was dissected and dissociated into single cells. Neurospheres can form from NSCs (green) or TAs, but not mature neurons (open circles). (*B*) The number of neurospheres that formed from the SVZ of a galectin-1-infused brain was greater than the number derived from control animals. * $p<0.05$. (*C*) The number of neural progenitor cells that fulfilled the criteria for NSCs (Sox21-positive and Dlx2-negative, see Fig. 1E) was greater in the galectin-1-infused brains than in control brains. * $p<0.05$. (*D*) The number of slowly dividing cells in *galectin-1*-knockout mice (KO) was lower than in wild-type mice (WT) [18], suggesting that galectin-1 is required for the maintenance of NSCs in the adult mouse brain. * $p<0.05$.

indicate that Gelectin-1 promotes the proliferation of adult NSCs.

The carbohydrate-biding ability of galectin-1 is required for its activity in the SVZ

Galectin-1 loses its carbohydrate-binding activity under oxidative conditions [9], but it gains other functions in the form of oxidized galectin-1, such as its involvement in functional recovery after peripheral nerve injury [37]. A mutant form of galectin-1, CS-galectin-1, in which the formation of disulfide bonds is prevented by substituting serines for cysteine residues, retains its carbohydrate-binding activity under oxidative conditions [38]. The infusion of CS-galectin-1, but not of oxidized-galectin-1, resulted in an increased number of long-term BrdU-retaining cells [18], suggesting that the carbohydrate-binding ability of galectin-1 is required to promote adult NSC proliferation.

Expression and function of glycan in NSCs

Our study suggests that the glycan expressed on NSCs functions in the proliferation of NSCs through its interactions with galectin-1. NSCs express a glycan structure, Le^X antigen [6, 39]. Le^X antigen is produced by the transference of fucose to lactosamine [39]. This addition of fucose significantly decreases the affinity of galectin-1 for lactosamine [10]. Future studies should reveal whether this fucosylation controls galectin-1 binding to NSCs, thereby regulating NSC proliferation.

Concluding remarks

To date, mechanisms that regulate endogenous adult NSCs have been extensively studied, with particular interest in their therapeutic potential [2, 40]. However, the functional significance of the interactions between the endogenous lectins and glycans expressed on adult NSCs has not been clarified. This study will contribute to our understanding of the importance of galectin-glycan interactions on adult NSCs, and to establishing novel approaches for treating human disease.

Acknowledgements. We thank Kazunobu Sawamoto, Françoise Poirier, Brian Key, Hisashi Narimatsu, Rikio Yabe and Jun Hirabayashi for valuable discussion, and Akiyo Hirayama, Michie Ito, Kayo Fujita, and Tomoko Uchida for secretarial assistance. This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT) (to H.O. and M.S); Solution oriented Research for Science and Technology of the Japan Society and Technology Agency (to H.O.); the 21st Century Centers of Excellence Program of MEXT (to Keio University), and a Naito Foundation Research Grant (to M.S).

1 Okano, H. (2002) Stem cell biology of the central nervous system. J. Neurosci. Res. 69, 698 – 707.
2 Okano, H. (2002) Neural stem cells: progression of basic research and perspective for clinical application. Keio J. Med. 51, 115 – 128.
3 Ming, G. L. and Song, H. (2005) Adult neurogenesis in the mammalian central nervous system. Annu. Rev. Neurosci. 28, 223 – 250.
4 Kempermann, G., Wiskott, L. and Gage, F. H. (2004) Functional significance of adult neurogenesis. Curr. Opin. Neurobiol. 14, 186 – 191.
5 Alvarez-Buylla, A., Seri, B. and Doetsch, F. (2002) Identification of neural stem cells in the adult vertebrate brain. Brain Res. Bull. 57, 751 – 758.
6 Capela, A. and Temple, S. (2002) LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. Neuron 35, 865 – 875.
7 Gates, M. A., Thomas, L. B., Howard, E. M., Laywell, E. D., Sajin, B., Faissner, A., Gotz, B., Silver, J. and Steindler, D. A. (1995) Cell and molecular analysis of the developing and adult mouse subventricular zone of the cerebral hemispheres. J. Comp. Neurol. 361, 249 – 266.
8 Doetsch, F. (2003) A niche for adult neural stem cells. Curr. Opin. Genet. Dev. 13, 543 – 550.

9 Leffler, H., Carlsson, S., Hedlund, M., Qian, Y. and Poirier, F. (2004) Introduction to galectins. Glycoconj. J. 19, 433 – 440.
10 Hirabayashi, J., Hashidate, T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, M., Urashima, T., Oka, T., Futai, M., Muller, W. E., Yagi, F. and Kasai, K. (2002) Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. Biochim. Biophys. Acta 1572, 232 – 254.
11 Camby, I., Le Mercier, M., Lefranc, F. and Kiss, R. (2006) Galectin-1: a small protein with major functions. Glycobiology 16, 137R – 157R.
12 Ivanova, N. B., Dimos, J. T., Schaniel, C., Hackney, J. A., Moore, K. A. and Lemischka, I. R. (2002) A stem cell molecular signature. Science 298, 601 – 604.
13 Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R. C. and Melton, D. A. (2002) "Stemness": transcriptional profiling of embryonic and adult stem cells. Science 298, 597 – 600.
14 Silva, W. A., Jr., Covas, D. T., Panepucci, R. A., Proto-Siqueira, R., Siufi, J. L., Zanette, D. L., Santos, A. R. and Zago, M. A. (2003) The profile of gene expression of human marrow mesenchymal stem cells. Stem Cells 21, 661 – 669.
15 Tumbar, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W. E., Rendl, M. and Fuchs, E. (2004) Defining the epithelial stem cell niche in skin. Science 303, 359 – 363.
16 Poirier, F., Chan, C. T., Timmons, P. M., Robertson, E. J., Evans, M. J. and Rigby, P. W. (1991) The murine H19 gene is activated during embryonic stem cell differentiation in vitro and at the time of implantation in the developing embryo. Development 113, 1105 – 1114.
17 Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97, 703 – 716.
18 Sakaguchi, M., Shingo, T., Shimazaki, T., Okano, H. J., Shiwa, M., Ishibashi, S., Oguro, H., Ninomiya, M., Kadoya, T., Horie, H., Shibuya, A., Mizusawa, H., Poirier, F., Nakauchi, H., Sawamoto, K. and Okano, H. (2006) A carbohydrate-binding protein, Galectin-1, promotes proliferation of adult neural stem cells. Proc. Natl. Acad. Sci. USA 103, 7112 – 7117.
19 Regan, L. J., Dodd, J., Barondes, S. H. and Jessell, T. M. (1986) Selective expression of endogenous lactose-binding lectins and lactoseries glycoconjugates in subsets of rat sensory neurons. Proc. Natl. Acad. Sci. USA 83, 2248 – 2252.
20 Poirier, F., Timmons, P. M., Chan, C. T., Guenet, J. L. and Rigby, P. W. (1992) Expression of the L14 lectin during mouse embryogenesis suggests multiple roles during pre- and post-implantation development. Development 115, 143 – 155.
21 Hynes, M. A., Gitt, M., Barondes, S. H., Jessell, T. M. and Buck, L. B. (1990) Selective expression of an endogenous lactose-binding lectin gene in subsets of central and peripheral neurons. J. Neurosci. 10, 1004 – 1013.
22 Akazawa, C., Nakamura, Y., Sango, K., Horie, H. and Kohsaka, S. (2004) Distribution of the galectin-1 mRNA in the rat nervous system: its transient upregulation in rat facial motor neurons after facial nerve axotomy. Neuroscience 125, 171 – 178.
23 Imbe, H., Okamoto, K., Kadoya, T., Horie, H. and Senba, E. (2003) Galectin-1 is involved in the potentiation of neuropathic pain in the dorsal horn. Brain Res. 993, 72 – 83.
24 Fukaya, K., Hasegawa, M., Mashitani, T., Kadoya, T., Horie, H., Hayashi, Y., Fujisawa, H., Tachibana, O., Kida, S. and Yamashita J. (2003) Oxidized galectin-1 stimulates the migration of Schwann cells from both proximal and distal stumps of transected nerves and promotes axonal regeneration after peripheral nerve injury. J. Neuropathol. Exp. Neurol. 62, 162 – 172.
25 Sango, K., Tokashiki, A., Ajiki, K., Horie, M., Kawano, H., Watabe, K., Horie, H. and Kadoya, T. (2004) Synthesis, localization and externalization of galectin-1 in mature dorsal root ganglion neurons and Schwann cells. Eur. J. Neurosci. 19, 55 – 64.
26 Kuchler, S., Joubert, R., Avellana-Adalid, V., Caron, M., Bladier, D., Vincendon, G. and Zanetta, J. P. (1989) Immunohistochemical localization of a beta-galactoside-binding lectin in rat central nervous system. II. Light- and electron-microscopical studies in developing cerebellum. Dev. Neurosci. 11, 414 – 427.
27 Joubert, R., Kuchler, S., Zanetta, J. P., Bladier, D., Avellana-Adalid, V., Caron, M., Doinel, C. and Vincendon, G. (1989) Immunohistochemical localization of a beta-galactoside-binding lectin in rat central nervous system. I. Light- and electron-microscopical studies on developing cerebral cortex and corpus callosum. Dev. Neurosci. 11, 397 – 413.
28 Puche, A. C. and Key, B. (1995) Identification of cells expressing galectin-1, a galactose-binding receptor, in the rat olfactory system. J. Comp. Neurol. 357, 513 – 523.
29 Mahanthappa, N. K., Cooper, D. N., Barondes, S. H. and Schwarting, G. A. (1994) Rat olfactory neurons can utilize the endogenous lectin, L-14, in a novel adhesion mechanism. Development 120, 1373 – 1384.
30 Horie, H., Inagaki, Y., Sohma, Y., Nozawa, R., Okawa, K., Hasegawa, M., Muramatsu, N., Kawano, H., Horie, M., Koyama, H., Sakai, I., Takeshita, K., Kowada, Y., Takano, M. and Kadoya, T. (1999) Galectin-1 regulates initial axonal growth in peripheral nerves after axotomy. J. Neurosci. 19, 9964 – 9974.
31 Puche, A. C., Poirier, F., Hair, M., Bartlett, P. F. and Key, B. (1996) Role of galectin-1 in the developing mouse olfactory system. Dev. Biol. 179, 274 – 287.
32 Sasaki, T., Hirabayashi, J., Manya, H., Kasai, K. I. and Endo, T. (2003) Galectin-1 induces astrocyte differentiation, which leads to production of brain-derived neurotrophic factor. Glycobiology 14, 357 – 363.
33 Morshead, C. M., Reynolds, B. A., Craig, C. G., McBurney, M. W., Staines, W. A., Morassutti, D., Weiss, S. and van der Kooy, D. (1994) Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron 13, 1071 – 1082.
34 Reynolds, B. A. and Weiss, S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707 – 1710.
35 Weiss, S., Reynolds, B. A., Vescovi, A. L., Morshead, C., Craig, C. G. and van der Kooy, D. (1996) Is there a neural stem cell in the mammalian forebrain? Trends Neurosci. 19, 387 – 393.
36 Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2002) EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. Neuron 36, 1021 – 1034.
37 Horie, H., Kadoya, T., Sango, K. and Hasegawa, M. (2005) Oxidized galectin-1 is an essential factor for peripheral nerve regeneration. Curr. Drug Targets 6, 385 – 394.
38 Hirabayashi, J. and Kasai, K. (1991) Effect of amino acid substitution by sited-directed mutagenesis on the carbohydrate recognition and stability of human 14-kDa beta-galactoside-binding lectin. J. Biol. Chem. 266, 23648 – 23653.
39 Sakaguchi, M., Sawamoto, K., Shimazaki, T., Kitamura, T., Shibuya, A. and Okano, H. (2005) A method for gene transfer, single isolation and *in vitro* assay for neural stem cells. Inflammation and Regeneration 25, 50 – 54.
40 Lindvall, O., Kokaia, Z. and Martinez-Serrano, A. (2004) Stem cell therapy for human neurodegenerative disorders-how to make it work. Nat. Med. 10 Suppl, S42 – 50.
41 Sawamoto, K., Wichterle, H., Gonzalez-Perez, O., Cholfin, J. A., Yamada, M., Spassky, N., Murcia, N. S., Garcia-Verdugo, J. M., Marin, O., Rubenstein, J. L., Tessier-Lavigne, M., Okano, H. and Alvarez-Buylla, A. (2006) New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 311, 629 – 632.