Biochemical and Biophysical Research Communications 463 (2015) 1091–1096

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

FGF-2 signal promotes proliferation of cerebellar progenitor cells and their oligodendrocytic differentiation at early postnatal stage

Masae Naruse, Koji Shibasaki, Yasuki Ishizaki*

Department of Molecular and Cellular Neurobiology, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan

ARTICLE INFO

Article history:
Received 28 May 2015
Accepted 8 June 2015
Available online 12 June 2015

Keywords:
Oligodendrocyte
Cerebellum
FGF-2
Neural stem cell
CD44
Development

ABSTRACT

The origins and developmental regulation of cerebellar oligodendrocytes are largely unknown, although some hypotheses of embryonic origins have been suggested. Neural stem cells exist in the white matter of postnatal cerebellum, but it is unclear whether these neural stem cells generate oligodendrocytes at postnatal stages. We previously showed that cerebellar progenitor cells, including neural stem cells, widely express CD44 at around postnatal day 3. In the present study, we showed that CD44-positive cells prepared from the postnatal day 3 cerebellum gave rise to neurospheres, while CD44-negative cells prepared from the same cerebellum did not. These neurospheres differentiated mainly into oligodendrocytes and astrocytes, suggesting that CD44-positive neural stem/progenitor cells might generate oligodendrocytes in postnatal cerebellum. We cultured CD44-positive cells from the postnatal day 3 cerebellum in the presence of signaling molecules known as mitogens or inductive differentiation factors for oligodendrocyte progenitor cells. Of these, only FGF-2 promoted survival and proliferation of CD44-positive cells, and these cells differentiated into O4+ oligodendrocytes. Furthermore, we examined the effect of FGF-2 on cerebellar oligodendrocyte development *ex vivo*. FGF-2 enhanced proliferation of oligodendrocyte progenitor cells and increased the number of O4+ and CC1+ oligodendrocytes in slice cultures. These results suggest that CD44-positive cells might be a source of cerebellar oligodendrocytes and that FGF-2 plays important roles in their development at an early postnatal stage.

1. Introduction

There are several hypotheses about the origin of cerebellar oligodendrocytes during embryonic stages. It has been reported that oligodendrocytes are derived from subependymal layers of the fourth ventricle and migrate into the cerebellum [1]. On the other hand, extracerebellar sources of cerebellar oligodendrocytes (the metencephalic vesicle and the parabasal plate of the mesencephalon) have been identified using chick brains [2,3]. Also, the analysis of *Ascl1* knockout mice suggests that the cerebellar oligodendrocyte progenitor cells (OPCs) have an extracerebellar origin and these OPCs invade the cerebellum during embryonic stages [4]. It has also been reported that neural stem cells exist in the white matter of the postnatal cerebellum, and a part of them express CD133 [5,6]. Detailed characteristics of these cerebellar neural stem cells, however, have not been elucidated yet. Furthermore, it is unclear if these cells give birth to oligodendrocytes, or how oligodendrocytic development is regulated in postnatal cerebellum.

Both platelet-derived growth factor (PDGF) and fibroblast growth factor-2 (FGF-2) independently regulate proliferation of OPCs in the forebrain and the spinal cord *in vitro* [7,8]. FGF-2 exerts important effects on proliferation, migration and differentiation of OPCs in culture, and converts mature oligodendrocytes into a novel phenotype [9–11]. FGF-2 was also reported to stimulate generation of oligodendrocytes from cultured cortical precursors [12] and from dorsally derived neural precursor cells of embryonic spinal cord and cerebral cortex [13–15]. Microinjection of FGF-2 into the lateral ventricle of E13.5 mouse embryo enhances proliferation of neuroepithelial cells, increases the number of OPCs generated from ganglionic eminence, and induces ectopic expression of OPCs in dorsal VZ [16]. Taken together, FGF-2 can act as a stimulator for production and proliferation of OPCs in the forebrain and the spinal cord. *Fgf2* is reportedly expressed in a few cells in the external

* Corresponding author.
E-mail address: yasukiishizaki@gunma-u.ac.jp (Y. Ishizaki).

http://dx.doi.org/10.1016/j.bbrc.2015.06.063
0006-291X/

granule cell layer at P0, in many cells in the internal granule cell layer at P7, and in Purkinje cells at P21 [17]. It is not known, however, if FGF-2 signaling affects oligodendrocyte development in postnatal cerebellum.

CD44 is a transmembrane glycoprotein important for cell–matrix adhesion and matrix-mediated cell signaling [18]. CD44 is known as a receptor for extracellular components such as hyaluronic acid [19]. It is also known that CD44 is expressed in astrocyte lineage cells in a dorsal domain of the rodent embryonic spinal cord [20,21], and at a low level, in astrocytes in the cortex and spinal cord [22–26]. In a previous study, we examined spatial and temporal expression profiles of CD44 expressing cells during development of mouse cerebellum by immunohistochemistry, *in situ* hybridization, and fluorescence-activated cell sorting (FACS), and showed that CD44 was expressed not only in astrocyte lineage cells but also in neural stem/progenitor cells and OPCs in cerebellum at early postnatal stages (P3, P7) [27].

In the present study, we have identified that CD44-positive cells in P3 cerebellum include neural stem/progenitor cells, and these cells proliferate and differentiate into oligodendrocytes in the presence of FGF-2 *in vitro*. We also showed that FGF-2 stimulates proliferation and differentiation of cerebellar OPCs *ex vivo*.

2. Materials and methods

2.1. Cell isolation by FACS

CD44-positive and CD44-negative cells were isolated as previously described [27]. Cerebella from P3 ICR mice were cut into small pieces and incubated at 37 °C for 10 min in trypsin solution (0.05% trypsin, 0.2 mM EDTA). The tissue was triturated in DPBS (Dulbecco's phosphate-buffered saline) containing 0.25 mg/ml trypsin inhibitor, 1.5 mg/ml BSA and 0.008% DNase I. Cells were suspended in 2%BSA/PBS at a density of 2×10^7 cells/mL and labeled with PE-conjugated anti-CD44 antibody for 30 min in the dark on ice. To remove dead cells, 7-amino-actinomycin D (BD Biosciences) was added to the cell suspension. CD44-positive and CD44-negative cells were sorted by using a FACSAria 2 flow cytometer (BD Biosciences).

2.2. Neurosphere assay

CD44-positive and CD44-negative cells were plated at 5 cells/μL in 24-well (0.5 mL/well) uncoated plates in serum-free medium [28] containing 20 ng/mL FGF-2 and 1 μg/mL heparin. To assess self-renewal, primary single neurosphere was subcloned by mechanically dissociating in serum-free medium containing FGF-2 and heparin. We assessed stem cell self-renewal by identifying new neurospheres after a further 7 days *in vitro*. To assess multipotentiality, single primary neurosphere colonies were transferred to individual wells of 8-well glass plates coated previously with Matrigel basement membrane matrix. Wells were processed 7 days later by use of immunocytochemistry.

2.3. CD44-positive cell culture

CD44-positive cells and CD44-negative cells isolated from whole cerebellum at P3 by FACS were plated at 4000 cells/well in 8-well glass plates coated by matrigel or 500 cells/well Terasaki microwell plates coated by matrigel in serum-free medium containing FGF-2 (20 ng/ml) and heparin (1 μg/ml), Shh (100 ng/ml), PDGF (20 ng/ml), or Forskolin (10 μM). The number of living cells in each microwell of Terasaki microwell plates was determined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTT) assay as described previously [29].

2.4. Slice culture

P3 mouse cerebella were cut into 350 μm parasagittal slices using a vibratome. Two to three slices were plated onto each

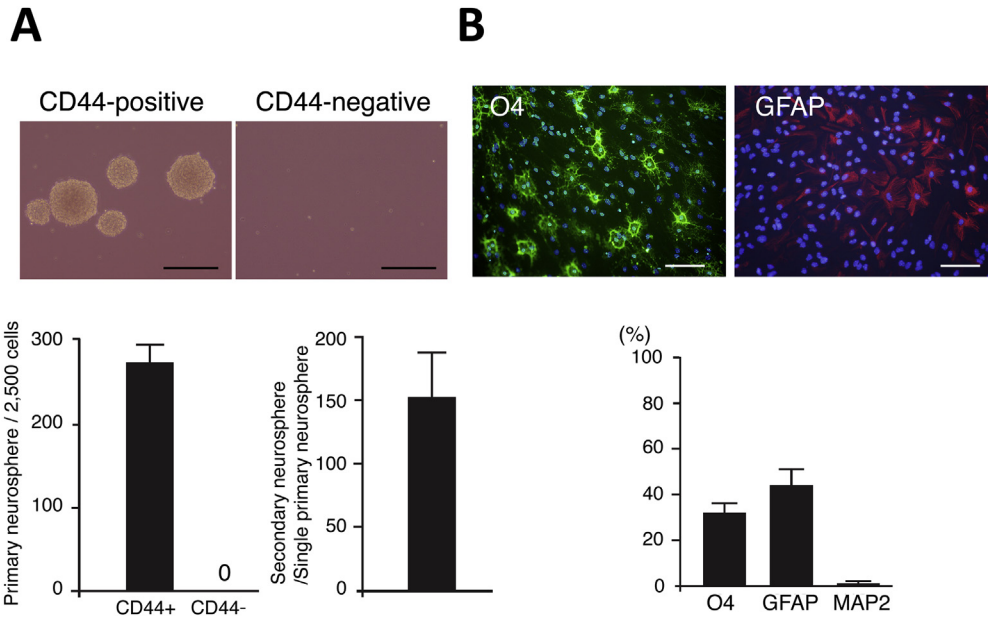

Fig. 1. CD44-positive cells yield neurospheres. CD44-positive cells and CD44-negative cells isolated from P3 cerebellum by FACS were cultured in serum free media with FGF-2 (Neurosphere assay). CD44-positive cells yield neurospheres (A). These primary neurospheres formed secondary neurospheres (A) and differentiated into oligodendrocytes, astrocytes and neurons (B). CD44-negative cells never yielded neurospheres (A). Data represent means ± SEM. Scale bars; 500 μm (A), 100 μm (B).

membrane insert, and the inserts were placed in a 6-well plate containing 1 mL serum-free medium contained FGF-2 (40 ng/ml) and heparin (1 μg/ml) or PDGF (40 ng/ml). These slices were cultured for 3 days. These slices were perfused by 4% PFA after 2 h BrdU (10 μM) treatment or 1 h O4 anti-body (kindly donated by Prof. K. Ono, Kyoto Pref. Univ. Med.) treatment, and then cut with a cryostat at a thickness of 18 μm.

2.5. Immunohistochemistry

Cells and tissue sections were incubated with 5% donkey serum in PBS-T for 1 h at room temperature, incubated with primary antibodies to cellular markers overnight at 4 °C, then incubated with secondary antibodies for 1 h at room temperature. The primary antibodies used included antibodies directed against BrdU (1:1000; Abcam), Ki67 (1:1000; BD Pharmingen), Olig2 (1:200; IBL), CC1 (1:400; Calbiochem), and GFAP (1:500; Millipore). For BrdU, Ki67 and Olig2 staining, the sections were incubated in citrate buffer by microwave before blocking buffer incubation.

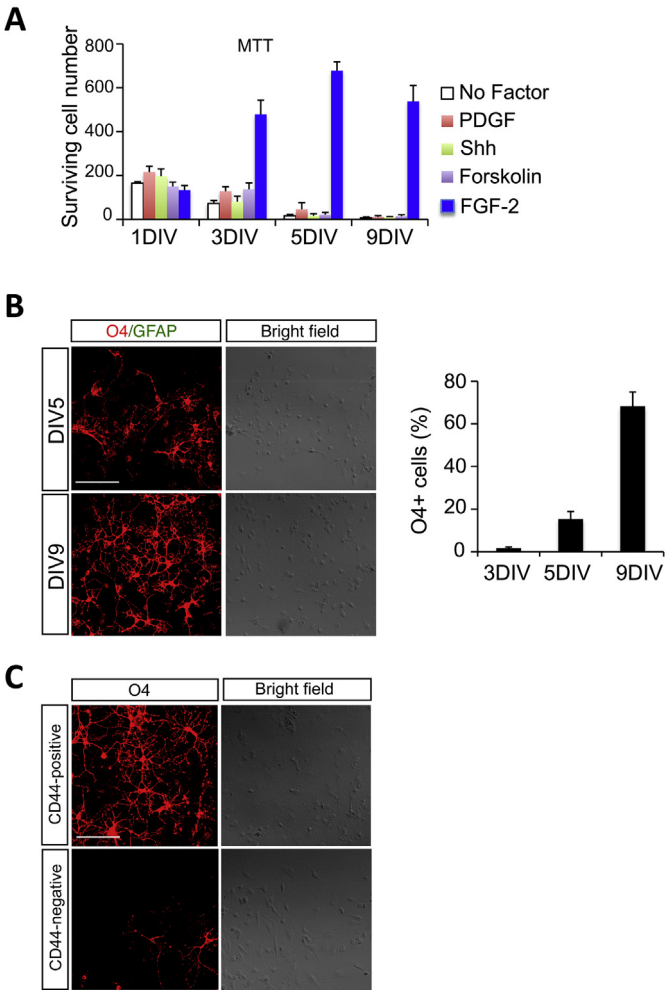

Fig. 2. FGF-2 promoted oligodendrocyte differentiation of CD44-positive cells. CD44-positive cells were cultured on matrigel coated plates with Shh, PDGF, Forskolin, and FGF-2. By MTT assay, the cell numbers were increased by FGF-2, but not PDGF, Shh and Forskolin (A). In addition, cells proliferated under FGF-2 differentiate into O4+ oligodendrocytes, but not GFAP + astrocytes for 9 days culture *in vitro* (9DIV) (B, C). On the other hand, the number of O4-positive oligodendrocyte differentiated from CD44-negative cells was very few even if under FGF-2 (C). Data represent means ± SEM. Scale bars; 100 μm (B and C).

3. Results and discussion

3.1. Cerebellar neural stem cells express CD44 at P3

Neural stem cells have been reported to exist in the white matter of postnatal cerebellum, and these neural stem cells have been isolated by their CD133 expression [5]. We suggested, however, that CD133-negative neural stem cells might exist in P3 cerebellum, because not only CD133-positive but also CD133-negative cells produced numerous neurospheres [28]. We previously reported that CD44 was expressed by Sox2+ neural stem cells in early postnatal mouse cerebellum [27]. We performed neurosphere assay to examine whether neural stem cells were included in CD44-positive cells. The fraction of CD44-positive cells was 27.3 ± 2.2% of the total single cells prepared from P3 cerebellum when sorted by FACS, and a part (approximately 10%) of CD44-positive cells yielded neurospheres (Fig. 1A). These primary neurospheres yielded secondary and tertiary neurospheres (Fig. 1A and data not shown). By contrast, CD44-negative cells isolated from P3 cerebellum never yielded neurospheres, suggesting that neural stem cells express CD44 in P3 cerebellum. Primary neurospheres produced by CD44-positive cells differentiated into oligodendrocytes, astrocytes and neurons (Fig. 1B). In the absence of signaling molecules, these neurospheres produced mainly astrocytes and oligodendrocytes, and only a few neurons. In the presence of retinoic acid, however, these neurospheres produced many MAP2+ neurons (data not shown). Taken together, primary neurospheres yielded from CD44-positive cells in P3 cerebellum had self-renewal and multipotential properties. We reported previously that EGF-responsive cells exist in P7 cerebellum. These cells formed neurospheres in the presence of EGF, and differentiated mainly into astrocytes (>90%) even in the presence of PDGF or retinoic acid [28]. These results suggest that the characteristics of neural stem cells in postnatal cerebellum might change depending on critical developmental periods.

3.2. FGF-2 promoted survival and oligodendrocytic differentiation of CD44-positive cells prepared from P3 cerebellum

We previously reported that cells expressing high levels of CD44 (CD44 ^{high} cells) prepared from early postnatal cerebellum are astrocyte progenitor cells [29]. CD44 ^{high} cells isolated from large cell fraction of P3 cerebellum were positive for a glial progenitor marker (A2B5), astrocyte lineage markers (BLBP, GLAST, vimentin), and a neural stem cell marker (nestin), but were negative for mature astrocyte markers (GFAP, S100β), neuronal marker (Tuj1, Pax6), or oligodendrocyte lineage markers (NG2, Olig2, O4). These cells died by apoptosis in the absence of any signaling molecule, and the surviving cells gradually expressed GFAP, indicating that differentiation into mature astrocytes is the default program for these cells. Treatment of these cells with LIF promoted their astrocytic differentiation. On the other hand, we also reported dynamic changes of CD44 expression in developing cerebellum [27]. CD44-positive cells (including not only highly expressing cells but also weakly expressing cells) isolated from whole cerebellum at P3 expressed nestin (>60%), GLAST (>80%), NG2 (>80%) and Olig2 (>40%), but not GFAP or O4 [27], suggesting that the main proportion of CD44-positive cells in P3 cerebellum might be neural stem/progenitor cells or glial progenitor cells. Histochemical analysis of CD44 expression in postnatal cerebellum showed that oligodendrocyte progenitor cells in P3 and P7 cerebellum temporarily expressed CD44 [27]. So we examined whether CD44-positive cells isolated from P3 cerebellum could differentiate into oligodendrocytes *in vitro* without neurosphere assay. All CD44-positive cells (including weakly expressing cells) isolated from P3 cerebellum were cultured with sonic hedgehog (Shh), PDGF,

forskolin, or FGF-2 (Fig. 2A), as these factors have been known as regulators of OPC proliferation/differentiation [7,8,15,30]. MTT assay revealed that FGF-2 increased the cell number (Fig. 2A). All other factors we examined did not maintain survival of these cells. These results show that FGF-2 signaling promotes survival and proliferation of CD44-positive cells from P3 cerebellum. Furthermore, FGF-2 promoted oligodendrocytic differentiation of these cells. After 9 days *in vitro* (9DIV), around 70% of cells expressed O4, but none of them expressed GFAP (Fig. 2B). On the other hand, CD44-negative cells cultured with FGF-2 survived but did not differentiate into O4+ oligodendrocytes (Fig. 2C). Taken together, FGF-2 promotes survival, proliferation, and oligodendrocytic differentiation of CD44-positive cells *in vitro*.

3.3. FGF-2 promoted proliferation of oligodendrocyte progenitor cells and increased the number of oligodendrocytes in cerebellar slice culture

To examine if FGF-2 affects development of oligodendrocytes in postnatal cerebellum *ex vivo*, P3 cerebellum slices were cultured with FGF-2. After 3 days *in vitro*, FGF-2 increased the number of Ki67+ or BrdU+ mitotic cells (Fig. 3A and B). In addition, FGF-2 treatment increased the number of Olig2+ cells and proliferative Olig2+ cells (Fig. 3B), suggesting FGF-2 promotes proliferation of OPCs in the developing cerebellum *ex vivo*. Hill and coworkers reported that PDGF increases the number of OPCs in the white matter of cortex and cerebellum, while FGF-2 didn't increase the number of OPCs in cortex [31]. The effect of FGF-2 on cerebellar OPCs was not examined in their report. In our cerebellar slice cultures, FGF-2 and PDGF increased the number of OPCs respectively (Fig. 3B). These combined results suggest that the responses to FGF-2 and PDGF of postnatal OPCs in corpus callosum and in the white matter of cerebellum might be different. FGF-2 also increased the number of O4+ or CC1+ oligodendrocytes in the white matter of our slice cultures (Fig. 4A and B), while FGF-2 reduced the intensity of GFAP expression in astrocytes (Fig. 4C). This indicates that FGF-2 supports oligodendrocytic, but not astrocytic differentiation in the developing cerebellum.

In this study, we show that neurospheres yielded by CD44-positive cells from P3 cerebellum differentiated into not only astrocytes but also oligodendrocytes *in vitro* (Fig. 1). It remains unknown if neural stem cells in the postnatal cerebellum produce oligodendrocytes, but there is a possibility that CD44-positive neural stem/progenitor cells in cerebellar white matter produce

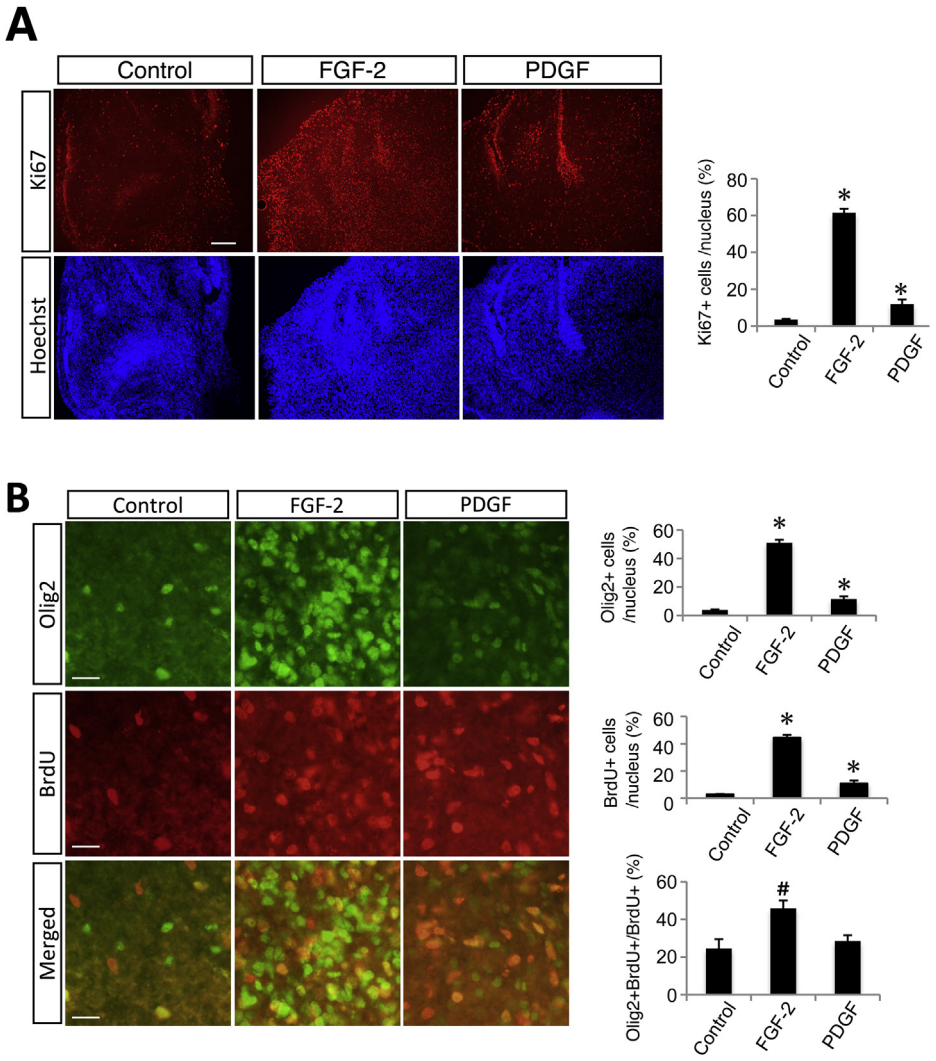

Fig. 3. FGF-2 promoted OPC proliferation in P3 cerebellar slice culture. P3 cerebellar slices were cultured under FGF-2 or PDGF for 3 days *in vitro*. The number of Ki67+ cells, BrdU+ cells and Olig2+ cells in white matter was counted. FGF-2 increased the number of Ki67+ cells compared with control (A). FGF-2 also increased the number of BrdU+ cells, Olig2+ cells and BrdU+/Olig2+ cells (B). PDGF also increased Ki67+ cells, BrdU+ cells and Olig2+ cells in P3 cerebellar slice culture. Data represent means ± SEM. $^{*}p < 0.001$, $^{\#}p < 0.005$. Scale bars; 200 μm (A), 20 μm (B).

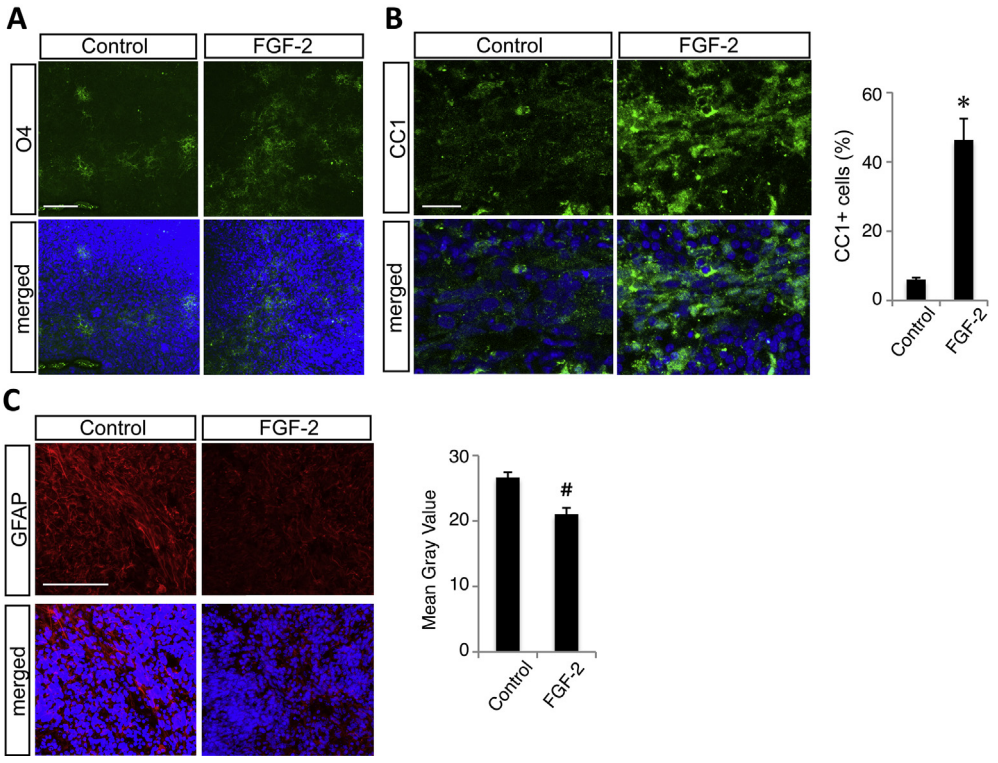

Fig. 4. FGF-2 increased the number of oligodendrocytes. P3 cerebellar slices were cultured under FGF-2 for 3 days *in vitro*. The number of O4+ cells and CC1+ cells in white matter increased with FGF-2 treatment (A and B). FGF-2 treatment decreased the staining intensity of GFAP (calculated using Image J) (C). Data represent means ± SEM. $^{*}p < 0.001$, $^{\#}p < 0.005$. Scale bars; 100 μm (A and C), 20 μm (B).

oligodendrocytes at around P3. Further careful analysis will be required to determine whether CD44-positive neural stem/progenitor cells give rise to oligodendrocytes and other type of cells *in vivo*. A part of CD44-positive cells in the dissociation culture survived and proliferated in the presence of FGF-2, but not in the presence of PDGF and other signaling molecules we examined. Surviving and proliferating CD44-positive cells differentiated into oligodendrocytes in the presence of FGF-2. These results suggest that FGF-2 effects are independent of other signaling molecules. PDGF increased the number of OPCs in cerebellar slice cultures (Fig. 3B), possibly through enhancing proliferation of OPCs, since PDGF does not promote proliferation of CD44-positive cells (neural stem/progenitor cells or glial progenitor cells, Fig. 2A). Although the origin of oligodendrocytes in the postnatal cerebellum and the *in vivo* developmental roles of FGF-2 still remain unknown, our study suggests that a portion of cerebellar oligodendrocytes might differentiate from CD44-positive cells in the early postnatal cerebellum under control of FGF signaling.

Acknowledgments

This work was supported by Grants-in-Aid for Science Research (Project No. 25122704 to Y. I.; 26111702 (Brain Environment), 26117502 (glial assembly) to K.S. and 15K18371 to M.N.) from the Japan Society for the Promotion of Science; by a Grant from the Strategic Research Program for Brain Sciences to Y. I.; by a Grant from Salt Science Research Foundation No. 14C2 to K.S.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.063.

References

[1] R. Reynolds, G.P. Wilkin, Development of macroglial cells in rat cerebellum. II. An in situ immunohistochemical study of oligodendroglial lineage from precursor to mature myelinating cell, Development 102 (1988) 409–425.
[2] K. Ono, H. Fujisawa, S. Hirano, M. Norita, T. Tsumori, et al., Early development of the oligodendrocyte in the embryonic chick metencephalon, J. Neurosci. Res. 48 (1997) 212–225.
[3] N. Mecklenburg, R. Garcia-Lopez, E. Puelles, C. Sotelo, S. Martinez, Cerebellar oligodendroglial cells have a mesencephalic origin, Glia 59 (2011) 1946–1957.
[4] P. Grimaldi, C. Parras, F. Guillemot, F. Rossi, M. Wassef, Origins and control of the differentiation of inhibitory interneurons and glia in the cerebellum, Dev. Biol. 328 (2009) 422–433.
[5] A. Lee, J.D. Kessler, T.A. Read, C. Kaiser, D. Corbeil, et al., Isolation of neural stem cells from the postnatal cerebellum, Nat. Neurosci. 8 (2005) 723–729.
[6] L. Zhang, J.E. Goldman, Generation of cerebellar interneurons from dividing progenitors in white matter, Neuron 16 (1996) 47–54.
[7] E.J. Collarini, N. Pringle, H. Mudhar, G. Stevens, R. Kuhn, et al., Growth factors and transcription factors in oligodendrocyte development, J. Cell. Sci. Suppl. 15 (1991) 117–123.
[8] E.E. Frost, J.A. Nielsen, T.Q. Le, R.C. Armstrong, PDGF and FGF2 regulate oligodendrocyte progenitor responses to demyelination, J. Neurobiol. 54 (2003) 457–472.
[9] D. Fortin, E. Rom, H. Sun, A. Yayon, R. Bansal, Distinct fibroblast growth factor (FGF)/FGF receptor signaling pairs initiate diverse cellular responses in the oligodendrocyte lineage, J. Neurosci. 25 (2005) 7470–7479.
[10] R. Bansal, Fibroblast growth factors and their receptors in oligodendrocyte development: implications for demyelination and remyelination, Dev. Neurosci. 24 (2002) 35–46.
[11] R. Bansal, S.E. Pfeiffer, FGF-2 converts mature oligodendrocytes to a novel phenotype, J. Neurosci. Res. 50 (1997) 215–228.
[12] X. Qian, A.A. Davis, S.K. Goderie, S. Temple, FGF2 concentration regulates the generation of neurons and glia from multipotent cortical stem cells, Neuron 18 (1997) 81–93.
[13] L. Gabay, S. Lowell, L.L. Rubin, D.J. Anderson, Deregulation of dorsoventral patterning by FGF confers trilineage differentiation capacity on CNS stem cells in vitro, Neuron 40 (2003) 485–499.
[14] S. Chandran, H. Kato, D. Gerreli, A. Compston, C.N. Svendsen, et al., FGF-dependent generation of oligodendrocytes by a hedgehog-independent pathway, Development 130 (2003) 6599–6609.
[15] N. Kessaris, F. Jamen, L.L. Rubin, W.D. Richardson, Cooperation between sonic hedgehog and fibroblast growth factor/MAPK signalling pathways in neocortical precursors, Development 131 (2004) 1289–1298.

[16] M. Naruse, E. Nakahira, T. Miyata, S. Hitoshi, K. Ikenaka, et al., Induction of oligodendrocyte progenitors in dorsal forebrain by intraventricular microinjection of FGF-2, Dev. Biol. 297 (2006) 262–273.
[17] Y. Yaguchi, T. Yu, M.U. Ahmed, M. Berry, I. Mason, et al., Fibroblast growth factor (FGF) gene expression in the developing cerebellum suggests multiple roles for FGF signaling during cerebellar morphogenesis and development, Dev. Dyn. 238 (2009) 2058–2072.
[18] D. Naor, S. Nedvetzki, I. Golan, L. Melnik, Y. Faitelson, CD44 in cancer, Crit. Rev. Clin. Lab. Sci. 39 (2002) 527–579.
[19] H. Ponta, L. Sherman, P.A. Herrlich, CD44: from adhesion molecules to signalling regulators, Nat. Rev. Mol. Cell. Biol. 4 (2003) 33–45.
[20] Y. Liu, S.S. Han, Y. Wu, T.M. Tuohy, H. Xue, et al., CD44 expression identifies astrocyte-restricted precursor cells, Dev. Biol. 276 (2004) 31–46.
[21] Y. Liu, Y. Wu, J.C. Lee, H. Xue, L.H. Pevny, et al., Oligodendrocyte and astrocyte development in rodents: an in situ and immunohistological analysis during embryonic development, Glia 40 (2002) 25–43.
[22] G. Moretto, R.Y. Xu, S.U. Kim, CD44 expression in human astrocytes and oligodendrocytes in culture, J. Neuropathol. Exp. Neurol. 52 (1993) 419–423.
[23] L. Alfei, M. Aita, B. Caronti, R. De Vita, V. Margotta, et al., Hyaluronate receptor CD44 is expressed by astrocytes in the adult chicken and in astrocyte cell precursors in early development of the chick spinal cord, Eur. J. Histochem. 43 (1999) 29–38.
[24] H. Akiyama, I. Tooyama, T. Kawamata, K. Ikeda, P.L. McGeer, Morphological diversities of CD44 positive astrocytes in the cerebral cortex of normal subjects and patients with Alzheimer's disease, Brain Res. 632 (1993) 249–259.
[25] N. Girgrah, M. Letarte, L.E. Becker, T.F. Cruz, E. Theriault, et al., Localization of the CD44 glycoprotein to fibrous astrocytes in normal white matter and to reactive astrocytes in active lesions in multiple sclerosis, J. Neuropathol. Exp. Neurol. 50 (1991) 779–792.
[26] T. Matsumoto, S. Imagama, K. Hirano, T. Ohgomori, T. Natori, et al., CD44 expression in astrocytes and microglia is associated with ALS progression in a mouse model, Neurosci. Lett. 520 (2012) 115–120.
[27] M. Naruse, K. Shibasaki, S. Yokoyama, M. Kurachi, Y. Ishizaki, Dynamic changes of CD44 expression from progenitors to subpopulations of astrocytes and neurons in developing cerebellum, PLoS One 8 (2013) e53109.
[28] T. Okano-Uchida, M. Naruse, T. Ikezawa, K. Shibasaki, Y. Ishizaki, Cerebellar neural stem cells differentiate into two distinct types of astrocytes in response to CNTF and BMP2, Neurosci. Lett. 552 (2013) 15–20.
[29] N. Cai, M. Kurachi, K. Shibasaki, T. Okano-Uchida, Y. Ishizaki, CD44-positive cells are candidates for astrocyte precursor cells in developing mouse cerebellum, Cerebellum 11 (2012) 181–193.
[30] D.W. Raible, F.A. McMorris, Induction of oligodendrocyte differentiation by activators of adenylate cyclase, J. Neurosci. Res. 27 (1990) 43–46.
[31] R.A. Hill, K.D. Patel, J. Medved, A.M. Reiss, A. Nishiyama, NG2 cells in white matter but not gray matter proliferate in response to PDGF, J. Neurosci. 33 (2013) 14558–14566.