FEBS Letters 583 (2009) 3643–3648

FEBS Letters

journal homepage: www.FEBSLetters.org

A novel neural-specific BMP antagonist, Brorin-like, of the Chordin family

Hiroyuki Miwa [1], Ayumi Miyake [1], Yuya Kouta, Akihiko Shimada, Yuki Yamashita, Yoshiaki Nakayama, Hajime Yamauchi, Morichika Konishi, Nobuyuki Itoh *

Department of Genetic Biochemistry, Kyoto University Graduate School of Pharmaceutical Sciences, Yoshida-shimoadachi, Sakyo, Kyoto 606-8501, Japan

ARTICLE INFO

Article history:
Received 16 September 2009
Revised 15 October 2009
Accepted 15 October 2009
Available online 21 October 2009

Edited by Ned Mantei

Keywords:
BMP
Antagonist
Chordin
Brain
Development
Gene expression

ABSTRACT

We identified a gene encoding a novel secreted protein in mice, humans, and zebrafish. As the protein of 222 amino acids is similar to Brorin, a secreted BMP antagonist, which is a member of the Chordin family, we named it Brorin-like. Recombinant Brorin-like protein weakly but significantly inhibited the activity of BMP in mouse preosteoblastic cells and promoted neurogenesis in mouse neural precursor cells. *Brorin-like* was predominantly expressed in the adult brain and embryonic neural tissues. The inhibition of *Brorin-like* functions in zebrafish resulted in the impairment of neural development. Brorin-like potentially plays roles in neural development and functions.

1. Introduction

Bone morphogenetic proteins (BMPs) are secreted signaling molecules belonging to the TGF-β superfamily [1]. *BMP* genes are expressed in embryonic tissues and organs. It has become apparent that BMP signaling is critical for morphogenesis and development [2,3]. *BMP* genes are also expressed in developing neural tissues [4]. BMPs alter the fate of neural precursor cells from neurogenesis to astrocytogenesis [5,6]. Additional observations that BMPs are also continuously expressed in the adult brain [7–10] led us to the notion that BMPs may be involved in adult neurogenesis.

The functions of BMPs are regulated by secreted regulators. Many secreted BMP regulators have been described. In vertebrates, such proteins include Noggin, the Chordin family, Follistatin, FSRP, and the DAN/ Cerberus family [3]. We previously reported *Brorin* encoding a novel secreted BMP antagonist identified in mice and humans [11]. Brorin with two cysteine-rich domains is a member of the Chordin family [12]. The *Brorin* gene is predominantly expressed in embryonic and adult neural tissues, and Brorin protein promotes neurogenesis in cultured mouse neural precursor cells [11]. We also report here the identification of a novel secreted BMP antagonist, Brorin-like, in mice, humans, and zebrafish. The amino acid sequence of Brorin-like is similar to that of Brorin. Brorin-like protein inhibited the activity of BMP and promoted neurogenesis like Brorin. Mouse *Brorin-like* was predominantly expressed in the neural tissues in adults and embryos like *Brorin*. The expression profile of *Brorin-like* is similar to but distinct from that of *Brorin*. The expression profile of *Brorin-like* is also similar to but distinct from that of *Brorin* in zebrafish embryos. The inhibition of *Brorin-like* functions in zebrafish resulted in the impairment of neural development. The present findings indicate that Brorin-like is a novel secreted BMP antagonist that potentially plays roles in neural development and functions.

2. Materials and methods

2.1. Identification of Brorin-like in mice and humans

Amino acid sequences predicted from mouse cDNAs of unknown function in nucleotide sequence databases were randomly analyzed using PSORT (Prediction of Protein Sorting Signals and Localization Sites in Amino Acid Sequences). cDNAs encoding putative secreted proteins were identified and cloned in a vector DNA, *pBluescript II SK* (+) (Stratagene). We named one of the cDNAs mouse *Brorin-like*. Human *Brorin-like* or zebrafish *Brorin-like* cDNA was also identified in a homology-based search of human or zebrafish cDNA sequences in nucleotide sequence databases, respectively.

* Corresponding author. Fax: +81 75 753 4600.
E-mail address: itohnobu@pharm.kyoto-u.ac.jp (N. Itoh).
[1] These authors contributed equally to this work.

0014-5793/$36.00
doi:10.1016/j.febslet.2009.10.044

2.2. Forced expression of mouse Brorin-like cDNA in COS-7 cells and insect cells

The mouse *Brorin-like* cDNA with a DNA fragment encoding a Myc tag and a His_6 tag at the 3′ terminus of the coding region was constructed between EcoRI and XbaI sites in a vector DNA, *pcDNA3.1*(+) (Invitrogen). COS-7 cells were transfected with the recombinant vector as described [11]. After the transfection, COS-7 cells were further cultured in fresh culture medium at 37 °C for 72 h. Brorin-like in the culture medium and lysate of the transfected cells was detected by Western blotting using an anti-Myc tag antibody (Cell Signaling) as described [11].

The coding region of mouse *Brorin-like* cDNA with a DNA fragment encoding a Myc tag and a His_6 tag at the 3′ terminus of the coding region was constructed in a transfer vector DNA, *pAcGP67A*. High Five cells infected with the recombinant baculovirus were cultured at 27 °C for 72 h in serum-free medium EX-CELL 405 (JRH Bioscience). Mouse Brorin-like was purified from the culture medium by affinity chromatography using Ni-NTA agarose as described [11]. Purified mouse Brorin-like was analyzed by Western blotting using an anti-Myc tag antibody as described [11].

2.3. Alkaline phosphatase activity in MC3T3-E1 cells

MC3T3-E1 cells were treated with human BMP2 or BMP6 (R&D systems), mouse Noggin (R&D systems) and mouse Brorin-like for 72 h. Alkaline phosphatase activity in the cells was determined as described [11]. Results are the means ± S.E.M. for 12 independent wells from four independent experiments.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Mouse or zebrafish cDNA was transcribed from the RNA with Molony murine leukemia virus reverse transcriptase. *Brorin-like* or *Brorin* cDNA was amplified from the cDNA with *Taq* DNA polymerase and primers specific for mouse *Brorin-like* or *Brorin* or zebrafish *Brorin-like*. Mouse β-*Actin* cDNA or zebrafish *elongation factor 1-α* (*elf1α*) cDNA was also amplified with primers specific for mouse β-*Actin* or zebrafish *elf1α* as a control. The amplified DNA was analyzed by agarose gel (1.5%) electrophoresis.

2.5. In situ hybridization

Mouse embryos and brain at postnatal day 56 (P56) were cut at 16 μm with a cryostat, as described. The sections were examined by in situ hybridization with a ^{35}S-labeled mouse antisense *Brorin-like* or *Brorin* RNA probe as described [11]. Whole-mount in situ hybridization was performed using zebrafish embryos, as previously described [20]. Zebrafish *Brorin-like* or *brorin* cDNA was cloned in a vector DNA, *pGEMT-T* (Clonetech). Digoxigenin-labeled *Brorin-like* and *Brorin* RNA probes were synthesized by in vitro transcription.

2.6. Neural differentiation activity in mouse neural precursor cells

Mouse neural precursor cells were prepared from the embryonic diencephalon at E13.5 as described [11]. The cells were treated with mouse Brorin-like or 10% FBS for 3 days. Neural differential activity in the neural precursor cell was determined by immunostaining using an anti-microtubule-associated protein 2 (MAP2) mouse monoclonal antibody (Sigma) or an anti-glial fibrillary acidic protein (GFAP) rabbit polyclonal antibody (Sigma) as described [11]. Results are the means ± S.E.M. for 20 different fields from four independent slides.

2.7. Morpholino oliogonucleotide injection into zebrafish embryos

The sequences of *Brorin-like* and universal control MOs used were as follows: a splice-site-targeted *Brorin-like* MO (MO1), the antisense sequence (25 bases) of which corresponds to that between the first intron and second exon of the coding region, 5′-CCATTCACAGGGCGAAGGCTGGG-3′; another splice-site-targeted *Brorin-like MO* (MO2), the antisense sequence (25 bases) of which corresponds to that between the first exon and intron of the coding region, 5′-CAGACAGCGTGTTCCTACCTTGAAC-3′; control MO (25 bases), 5′-CCTCTTACCTCAGTTACAATTTATA-3′. *Brorin-like* MO1 (2 ng), *Brorin-like* MO2 (4 ng) or control MO (2 or 4 ng) were injected into four central blastomeres of 16-cell embryos. To determine its efficacy, *Brorin-like* MO1 (8 ng) or *Brorin-like* MO2 (16 ng) was injected into 2-cell embryos. RNA was isolated from wild-type, *Brorin* MO1-injected or *Brorin* MO2-injected embryos at 24 hpf. *Brorin-like* cDNA and *ef1α* cDNA as a control were amplified from the RNA by RT-PCR, as described above.

2.8. Statistical analysis

Data are expressed as means ± S.E.M. The significance of differences in mean values was assessed using Student's *t*-test.

3. Results and discussion

3.1. Identification of mouse and human Brorin-like

We identified mouse cDNA encoding a novel putative secreted protein of 222 amino acids with a putative signal sequence (21 amino acids) at its amino-terminus and two cysteine-rich domains in its core region (GenBank AB374230) (Fig. 1A). As the protein is significantly similar (48.6% amino acid identity) to mouse Brorin (324 amino acids), a secreted BMP antagonist [11], we named it mouse Brorin-like. Human *Brorin-like c*DNA (AB374231) was also identified. The amino acid sequence of human Brorin-like (222 amino acids) was highly similar (98.6% identity) to that of mouse Brorin-like (Fig. 1A).

3.2. Comparison of Brorin-like with members of the Chordin family

The Chordin family, a group of proteins with cysteine-rich domains that consist of 10 cysteine residues, comprises several members, Brorin, Chordin, Chordin-like 1/Neuralin/Neurogenesin-1 (CHRDL1), Chordin-like 2 (CHRDL2), and Crossveinless 2 [11–16]. Brorin, Chordin, CHRDL1, and CHRDL2 are BMP antagonists; however, Crossveinless 2 functions as a BMP antagonist and a pro-BMP factor. As Brorin-like is very similar to Brorin, Brorin-like is also a member of the Chordin family. Members of the Chordin family have different numbers (two, three, or four) of cysteine-rich domains (Fig. 1B). Brorin and Brorin-like have two sets of cysteine-rich domains, and other members have three or four sets, indicating that Brorin and Brorin-like are distinct from any other member of the Chordin family.

3.3. Forced expression of mouse Brorin-like cDNA in cultured cells, COS-7 cells

Mouse cDNA encoding *Brorin-like* was expressed in cultured cells, COS-7 cells. Both the culture medium and cell lysate were examined by Western blotting. Three major bands of ~40, 39, and 36 kDa were detected in the culture medium, indicating that Brorin-like is a secreted protein (Fig. 2A). The observed molecular masses were larger than the calculated molecular mass of the

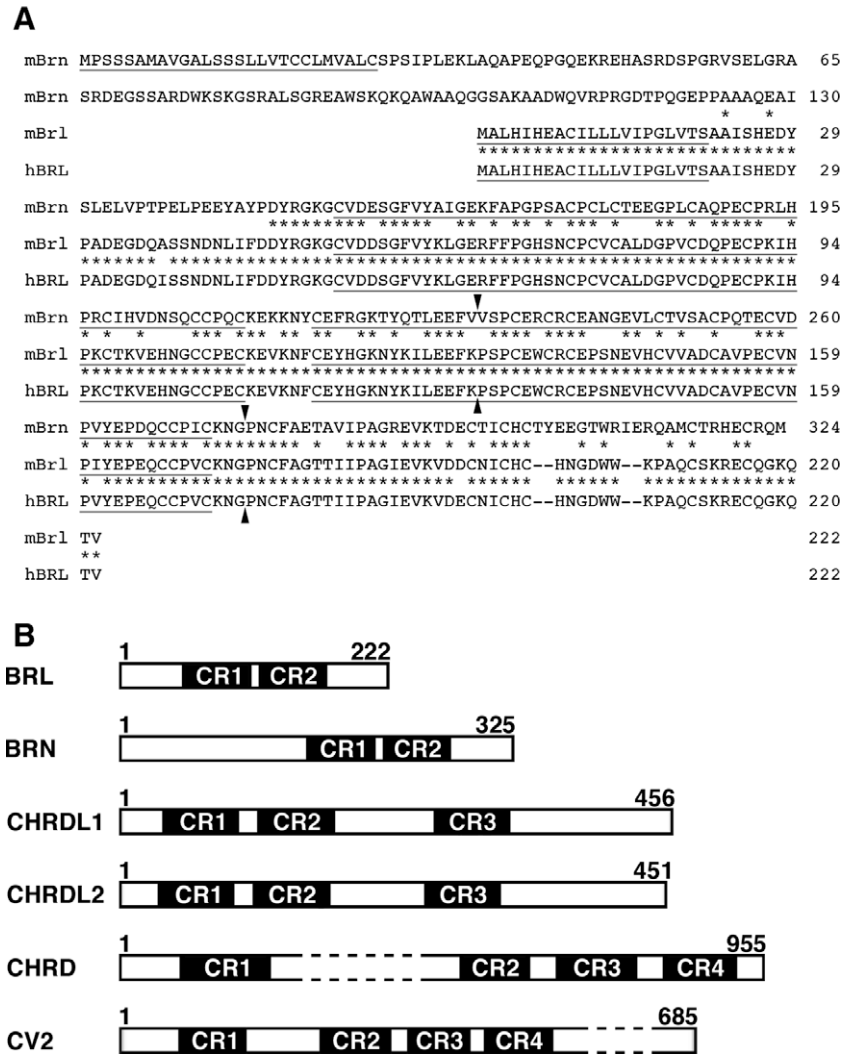

Fig. 1. Structure of Brorin-like and schematic representation of human Chordin family members. (A) Comparison of amino acid sequences of mouse Brorin-like (mBrl), human Brorin-like (hBRL), and mouse Brorin (mBrn). Underlines at the amino-termini and core sequences indicate putative secreted signal sequences and cysteine-rich domains, respectively. Arrowheads indicate the positions of introns. (B) Comparison of cysteine-rich domain positions of human Chordin family members, Brorin-like (BRL), Brorin (BRN), Chordin-like 1 (CHRDL1), Chordin-like 2 (CHRDL2), Chordin (CHRD), and Crossveinless 2 (CV2). CR1, CR2, CR3, and CR4 indicate cysteine-rich domains.

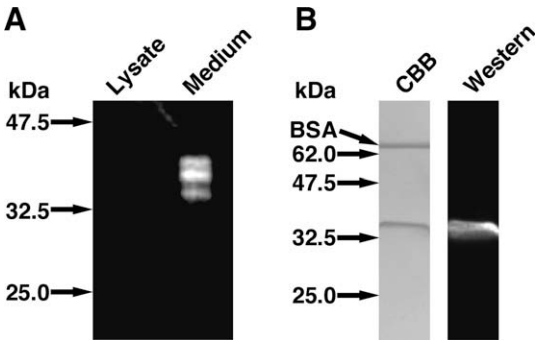

Fig. 2. Detection of mouse Brorin-like protein. (A) The lysate and culture medium of cells expressing mouse *Brorin-like* cDNA were analyzed by Western blotting. Three major bands (~40, 39 and 36 kDa) of mouse Brorin-like protein were detected. (B) Mouse Brorin-like protein was purified from the culture medium of High Five cells expressing mouse *Brorin-like* cDNA. Purified Brorin-like protein with BSA as a carrier was separated by SDS–polyacrylamide gel electrophoresis. The protein was detected by protein staining with Coomassie Brilliant Blue R-250 (CBB) and by Western blotting (Western).

recombinant Brorin-like protein (25 010 Da), indicating Brorin-like to be subjected to post-translational modification.

3.4. Production of purified mouse Brorin-like protein

To prepare purified mouse Brorin-like protein, mouse *Brorin-like* cDNA was expressed in cultured High Five insect cells by infection with a recombinant baculovirus containing the mouse *Brorin-like* cDNA. Mouse Brorin-like was purified from the culture medium by affinity chromatography. Purified Brorin-like was separated by SDS–polyacrylamide gel electrophoresis followed by protein staining. Purified Brorin-like showed a single band of ~35 kDa with a band of bovine serum albumin (BSA) (66 kDa) as a carrier (Fig. 2B). Purified Brorin-like also showed a single band of ~35 kDa by Western blotting.

3.5. Effects of Brorin-like protein on BMP signaling in MC3T3-E1 cells

Brorin is a BMP antagonist, indicating that Brorin-like might be also. BMPs are signaling molecules for the stimulation of osteoblastic differentiation [17]. Most are members of the dpp and 60A subgroups of the TGF-β superfamily [4]. We examined the effect of mouse Brorin-like and mouse Noggin as a control of a BMP antagonist on the activity of BMP2 and BMP6, as representatives of the dpp and 60A subgroups, respectively, for the differentiation of

MC3T3-E1 cells by determining alkaline phosphatase activity [18]. Although the alkaline phosphatase activity stimulated by BMP2 or BMP6 was strongly inhibited by Noggin, the activity was weakly but significantly inhibited by Brorin-like (Fig. 3A). BMPs also induce the phosphorylation of Smad. BMP2 and BMP6 induced the phosphorylation of Smad1/5/8 in MC3T3-E1 cells. The phosphorylation induced by BMP2 or BMP6 was strongly inhibited by Noggin. However, the phosphorylation was not significantly inhibited by Brorin-like (data not shown).

3.6. Expression of Brorin-like in mouse embryos and adult tissues

The expression of *Brorin-like* in mouse embryos during E12.5–E18.5 was examined by in situ hybridization (Fig. 4Aa–d). Expression was significant in the developing neural tissues, including several discrete regions in the forebrain, midbrain, and hindbrain, and the spinal cord. The expression profiles of *Brorin-like* in embryos are similar to but distinct from those of *Brorin* (Fig. 4Ae–h) [11]. The expression of *Brorin-like* in mouse tissues at P56 was examined by RT-PCR. The expression of *Brorin-like* was predominantly detected in the brain among the major organs examined (Fig. 4B). The brain-specific expression profile is similar to that of *Brorin* (Fig. 4B) [11]. The expression of *Brorin-like* in the brain at P56 was also examined by in situ hybridization. Expression was widely detected in the brain (Fig. 4Ca–f). However, prominent expression of *Brorin-like* was observed in several discrete regions including the thalamus, hypothalamus, and hippocampus. The expression profiles of *Brorin-like* in the brain are also similar to but distinct from those of *Brorin* (Fig. 4Cg–l) [11]. These results indicate that Brorin-like has unique roles in developing and adult neural tissues.

3.7. Effects of Brorin-like on neuronal and astrocytic differentiation in cultured neural precursor cells

As the expression profiles of *Brorin-like* indicated its potential roles in neural differentiation, we examined the effect of mouse Brorin-like and FBS (10%), which promotes neural differentiation in neural precursor cells [19], as a control on the neural differentiation of mouse neural precursor cells. After the neural precursor cells had been cultured for 3 days, the differentiation was examined by double immunostaining using anti-MAP2 and GFAP antibodies for neurons and astrocytes, respectively. FBS increased the proportion of neurons and generated astrocytes among the neural precursor cells (Fig. 3B). Brorin-like also increased the proportion of neurons among the neural precursor cells. However, treatment with Brorin-like did not produce astrocytes. These results indicate that Brorin-like induced neurogenesis but not astrogenesis.

3.8. Expression and functions of Brorin-like in zebrafish embryos

As the functions of zebrafish genes can be effectively blocked by antisense morpholino oligonucleotides in vivo, the zebrafish is a useful model to elucidate the functions of genes in vertebrates in vivo [21]. We identified zebrafish DNA encoding Brorin-like (AB428728), of which the amino acid sequence (223 amino acids) is significantly similar (~78% identity) to that of mouse Brorin-like (data not shown). We examined the expression of *Brorin-like* in zebrafish embryos at 36 hpf by whole-mount in situ hybridization. The expression of *Brorin-like* was predominantly detected in the embryonic brain, including several discrete regions in the forebrain, midbrain, and hindbrain (Fig. 5Aa and b). The neural tissue-specific expression profile of *Brorin-like* in zebrafish embryos

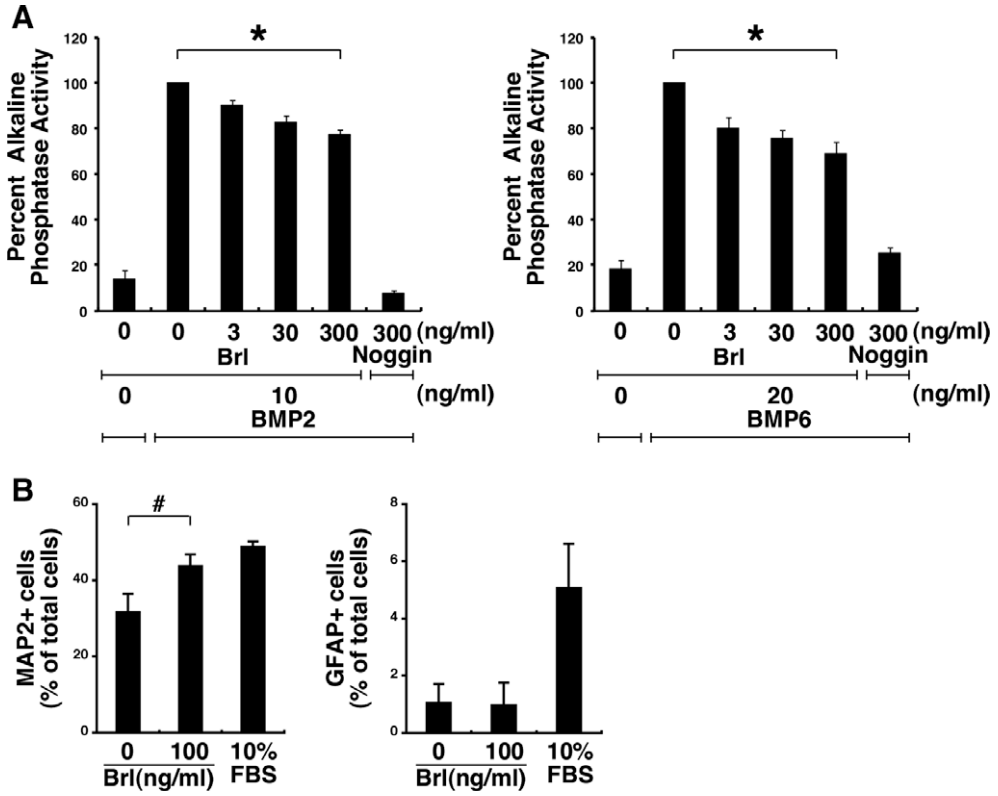

Fig. 3. Effects of mouse Brorin-like protein on alkaline phosphatase activity in MC3T3-E1 cells induced by BMP and neuronal and astrocytic differentiation in mouse neural precursor cells. (A) The alkaline phosphatase activity in MC3T3-E1 cells was measured. Results are the means ± S.E.M. for 12 independent wells from four independent experiments ($^{*}P < 0.02$). (B) The neural precursor cells were examined by immunochemistry. Neuronal or astrocytic differentiation was qualified by counting MAP2-positive cells or GFAP-positive cells, respectively. Results are the means ± S.E.M. for 20 different fields from four independent slides ($^{\#}P < 0.07$). Brorin-like; Brl.

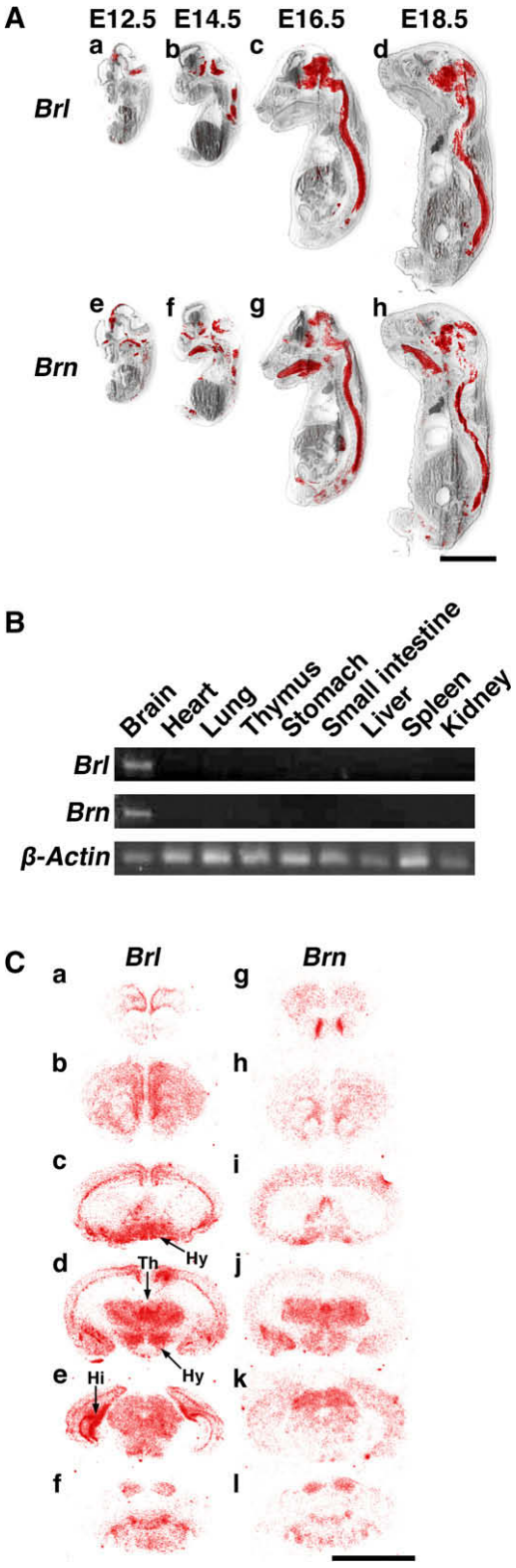

Fig. 4. Expression of *Brorin-like* and *Brorin* in mouse embryos and adult tissues and brains. (A) Sagittal sections of mouse embryos were examined by in situ hybridization. Red grains superimposed upon a hematoxylin-eosin stain show the localization of *Brorin-like* (a–d) or *Brorin* (e–h) mRNA. *Brl*: *Brorin-like, Brn*: *Brorin*. Scale bar = 5 mm. (B) The expression of *Brorin-like* or *Brorin* in mouse adult tissues at P56 was examined by RT-PCR. The expression of β-*Actin* was also examined as a control. *Brl*: *Brorin-like, Brn*: *Brorin*. (C) Coronal sections of mouse brain at P56 were examined by in situ hybridization. Red grains show the localization of *Brorin-like* (a–f) or *Brorin* (g–l) mRNA. Th: thalamus, Hy: hypothalamus, Hi: hippocampus. *Brl*; *Brorin-like, Brn*; *Brorin*. Scale bar = 5 mm.

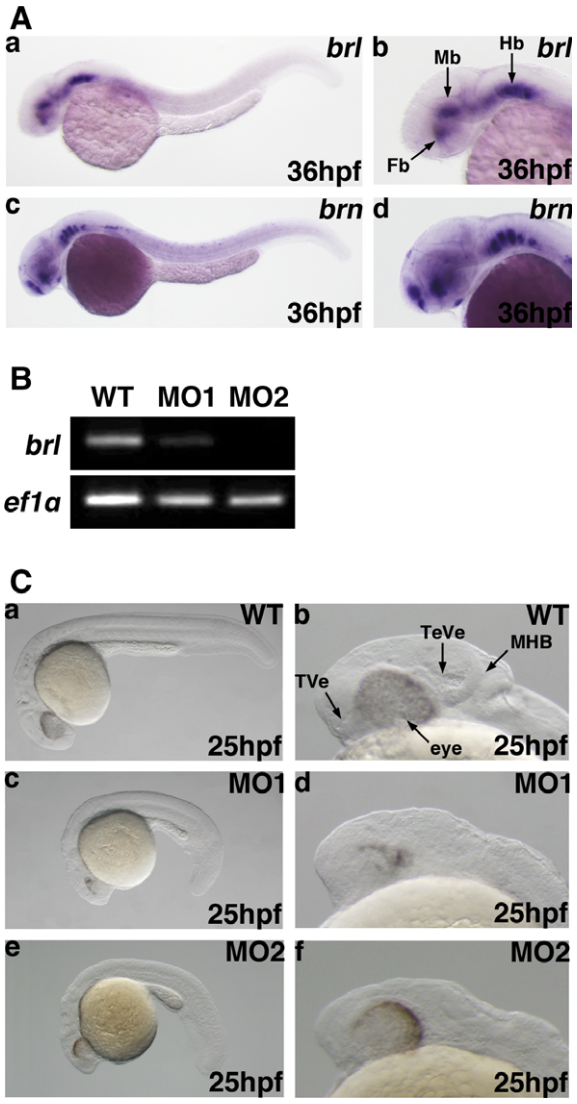

Fig. 5. Expression and functions of *Brorin-like* in zebrafish embryos. (A) The expression of *Brorin-like* (a, b) or *Brorin* (c, d) in zebrafish embryos at 36 hpf was examined by whole-mount in situ hybridization. The localization of *Brorin-like* or *Brorin* mRNA is shown in blue. Fb: forebrain, Mb: midbrain, Hb, hindbrain. *Brl*: *Brorin-like, brn*: *Brorin*. (B) *Brorin-like* cDNA was amplified from wild-type, *Brorin* MO1-injected or *Brorin* MO2-injected embryonic cDNA by RT-PCR followed by agarose gel (1.5%) electrophoresis. The gel was stained with ethidium bromide. *ef1α* cDNA was also amplified as a control. *Brl*: *Brorin-like*. (C) Lateral views of wild-type (a, b), *Brorin-like* MO1-injected (c, d), or *Brorin-like* MO2-injected (e, f) zebrafish embryos at 25 hpf are shown. Wild-type embryos developed normally. Neural development in the brain and eye in *Brorin-like* MO1- or MO2-injected zebrafish embryos was impaired. TVe: telencephalic ventricle, TeVe: tectal ventricle, MHB: midbrain-hindbrain boundary. WT: wild-type.

is essentially consistent with that of *Brorin-like* in mouse embryos (Fig. 4A). We also identified zebrafish *Brorin* cDNA (AB292672) (unpublished data), and examined its expression. The expression profile of *Brorin-like* was similar to but slightly distinct from that of *Brorin* (Fig. 5Ac and d).

To inhibit *Brorin-like* functions in zebrafish embryos, we injected spliced-site-targeted *Brorin-like* MOs, MO1 and MO2, the sequences of which correspond to different boundary regions between the intron and exon of the coding region, into zebrafish embryos. We examined whether *Brorin-like* MOs could efficiently block the splicing of the *Brorin-like* mRNA precursor. The expression of mature *Brorin* mRNA examined by RT-PCR was markedly decreased in MO-injected embryos (Fig. 5B). These results indicate

that *Brorin-like* MOs effectively blocked the maturation of *Brorin-like* mRNA. To examine the roles of *Brorin-like* in zebrafish neural development, we injected *Brorin-like* MO1 into four central blastomeres of 16-cell embryos. The MO1-injected embryos at 25 hpf showed morphological abnormalities in the brain and eye ($n = 71/96$) (Fig. 5Cc and d). In the *Brorin-like* knockdown brain, the formations of the telencephalic and tectal ventricles were apparently impaired. The formation of the constriction between the midbrain and hindbrain was also impaired. The *Brorin-like* MO2-injected embryos also showed similar morphological abnormalities ($n = 20/32$) (Fig. 5Cc and d). In contrast, universal control MO-injected embryos did not show any morphological abnormalities (data not shown). These results indicate that Brorin-like plays crucial roles in neural development. We also injected *Brorin* MO1 (a spiced-site-targeted MO) or MO2 (a translation initiation-site-targeted MO) into zebrafish embryos (unpublished observations). The *Brorin* knockdown embryos also showed morphological abnormalities in the brain and eye. However, the phenotype of *Brorin* knockdown embryos was apparently severer than that of *Brorin-like* knockdown embryos. The formations of the telencephalic and tectal ventricles and constriction between the midbrain and hindbrain were apparently impaired in the embryos. Furthermore, the embryos exhibited a significant reduction in the size of the brain (unpublished observations). As the phenotype of *Brorin-like* knockdown embryos is similar to but distinct from that of *Brorin* knockdown embryos, Brorin-like potentially plays roles which are similar to but distinct from those of Brorin, in neural development.

Acknowledgements

This work was supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan.

References

[1] Kingsley, D.M. (1994) The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev. 8, 133–146.
[2] von Bubnoff, A. and Cho, K.W. (2001) Intracellular BMP signaling regulation in vertebrates: pathway or network? Dev. Biol. 239, 1–14.
[3] Balemans, W. and Van Hul, W. (2002) Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators. Dev. Biol. 250, 231–250.
[4] Lein, P., Drahushak, K.M. and Higgins, D. (2002) Bone morphogenetic proteins: from laboratory to clinical practice. Birkhauser Verlag, Basel, pp. 289–319.
[5] Nakashima, K., Takizawa, T., Ochiai, W., Yanagisawa, M., Hisatsune, T., Nakafuku, M., Miyazono, K., Kishimoto, T., Kageyama, R. and Taga, T. (2001) BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis. Proc. Natl. Acad. Sci. USA 98, 5868–5873.
[6] Yanagisawa, M., Takizawa, T., Ochiai, W., Uemura, A., Nakashima, K. and Taga, T. (2001) Fate alteration of neuroepithelial cells from neurogenesis to astrocytogenesis by bone morphogenetic proteins. Neurosci. Res. 41, 391–396.
[7] Mehler, M.F., Mabie, P.C., Zhang, D. and Kessler, J.A. (1997) Bone morphogenetic proteins in the nervous system. Trends Neurosci. 20, 309–317.
[8] Ebendal, T., Bengtsson, H. and Soderstrom, S. (1998) Bone morphogenetic proteins and their receptors: potential functions in the brain. J. Neurosci. Res. 51, 139–146.
[9] Charytoniuk, D.A., Traiffort, E., Pinard, E., Issertial, O., Seylaz, J. and Ruat, M. (2000) Distribution of bone morphogenetic protein and bone morphogenetic protein receptor transcripts in the rodent nervous system and up-regulation of bone morphogenetic protein receptor type II in hippocampal dentate gyrus in a rat model of global cerebral ischemia. Neuroscience 100, 33–43.
[10] Chen, H.L., Lein, P.J., Wang, J.Y., Gash, D., Hoffer, B.J. and Chiang, Y.H. (2003) Expression of bone morphogenetic proteins in the brain during normal aging and in 6-hydroxydopamine-lesioned animals. Brain Res. 994, 81–90.
[11] Koike, N., Kassai, Y., Kouta, Y., Miwa, H., Konishi, M. and Itoh, N. (2007) Brorin, a novel secreted bone morphogenetic protein antagonist, promotes neurogenesis in mouse neural precursor cells. J. Biol. Chem. 282, 15843–15850.
[12] Garcia Abreu, J., Coffinier, C., Larrain, J., Oelgeschlager, M. and De Robertis, E.M. (2002) Chordin-like CR domains and the regulation of evolutionarily conserved extracellular signaling systems. Gene 287, 39–47.
[13] Ueki, T., Tanaka, M., Yamashita, K., Mikawa, S., Qiu, Z., Maragakis, N.J., Hevner, R.F., Miura, N., Sugimura, H. and Sato, K. (2003) A novel secretory factor, Neurogenesin-1, provides neurogenic environmental cues for neural stem cells in the adult hippocampus. J. Neurosci. 23, 11732–11740.
[14] Chandra, A., Itakura, T., Yang, Z., Tamakoshi, T., Xue, X., Wang, B., Ueki, T., Sato, K., Uezato, T. and Miura, N. (2006) Neurogenesin-1 differentially inhibits the osteoblastic differentiation by bone morphogenetic proteins in C2C12 cells. Biochem. Biophys. Res. Commun. 344, 786–791.
[15] Coffinier, C., Ketpura, N., Tran, U., Geissert, D. and De Robertis, E.M. (2002) Mouse Crossveinless-2 is the vertebrate homolog of a Drosophila extracellular regulator of BMP signaling. Mech. Dev. 119, S179–S184.
[16] Ikeya, M., Kawada, M., Kiyonari, H., Sasai, N., Nakao, K., Furuta, Y. and Sasai, Y. (2006) Essential pro-Bmp roles of crossveinless 2 in mouse organogenesis. Development 133, 4463–4473.
[17] Hogan, B.L. (1996) Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev. 10, 1580–1594.
[18] Franceschi, R.T., Iyer, B.S. and Cui, Y. (1994) Effects of ascorbic acid on collagen matrix formation and osteoblast differentiation in murine MC3T3-E1 cells. J. Bone Miner. Res. 9, 843–854.
[19] Brunet, J.F., Grollimund, L., Chatton, J.Y., Lengacher, S., Magistretti, P.J., Villemure, J.G. and Pellerin, L. (2004) Early acquisition of typical metabolic features upon differentiation of mouse neural stem cells into astrocytes. Glia 46, 8–17.
[20] Koshida, S., Shinya, M., Mizuno, T., Kuroiwa, A. and Takeda, H. (1998) Initial anteroposterior pattern of the zebrafish central nervous system is determined by differential competence of the epiblast. Development 125, 1957–1966.
[21] Shin, J.T. and Fishman, M.C. (2002) From Zebrafish to human: modular medical models. Annu. Rev. Genom. Hum. Genet. 3, 311–340.