Brorin, a Novel Secreted Bone Morphogenetic Protein Antagonist, Promotes Neurogenesis in Mouse Neural Precursor Cells*

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We identified a gene encoding a novel secreted protein in mice and humans and named it Brorin. Mouse Brorin consists of 324 amino acids with a putative secreted signal sequence at its amino terminus and two cysteine-rich domains in its core region. Positions of 10 cysteine residues in the domains of Brorin are similar to those in the cysteine-rich domains of members of the Chordin family. However, the amino acid sequence of Brorin is not significantly similar to that of any other member of the Chordin family, indicating that Brorin is a unique member of the family. Mouse Brorin protein produced in cultured cells was efficiently secreted into the culture medium. The protein inhibited the activity of bone morphogenetic protein 2 (BMP2) and BMP6 in mouse preosteoblastic MC3T3-E1 cells. Mouse Brorin was predominantly expressed in neural tissues in embryos and also predominantly expressed in the adult brain. In the brain, the expression was detected in neurons, but not glial cells. The neural tissue-specific expression profile of Brorin is quite distinct from that of any other member of the Chordin family. Brorin protein promoted neurogenesis, but not astrogenesis, in mouse neural precursor cells. The present findings indicate that Brorin is a novel secreted BMP antagonist that potentially plays roles in neural development and functions.

Bone morphogenetic proteins (BMPs) 2 are secreted signaling molecules belonging to the TGFβ superfamily (1). BMPs regulate embryonic development in most tissues and organs. It has become apparent that the fine-tuning of BMP signaling is critical for a variety of their functions, for example, in neural induction, limb morphogenesis, and skeletal development (2, 3). The functions of BMPs are regulated by secreted regulators. An increasing number of secreted BMP regulators have been described. In vertebrates, such proteins include Noggin, the Chordin family, Follistatin, FSRP, and the DAN/Cerberus family (3).

BMPs 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.

Many secreted signaling proteins, including BMPs, FGFs, Wnts, and their regulators, play crucial roles in cell proliferation and differentiation. The identification and characterization of novel secreted signaling proteins are expected to provide new insights into the mechanism of cell proliferation and differentiation. We identified many genes encoding novel secreted proteins from mouse cDNAs of unknown function in the DNA databases. We termed one of them Brorin. Brorin has two cysteine-rich domains. The positions of cysteine in the domains are similar to those of members of the Chordin family that are secreted BMP regulators (11). We report here the identification of a novel secreted BMP antagonist, Brorin, which was predominantly expressed in the neural tissues in mouse adults and embryos and promoted neurogenesis in mouse neural precursor cells. Although its physiological role remains to be elucidated, Brorin is expected to play roles in neural development and functions.

EXPERIMENTAL PROCEDURES

Identification of Mouse and Human Brorin—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) (psort.im.s.u-tokyo.ac.jp/form2.html). Many cDNAs encoding putative secreted proteins were identified. We isolated full-length cDNAs by polymerase chain reaction (PCR) with the embryonic cDNA (embryonic day 14.5, E14.5) as a template and cloned them in a vector DNA, pBluescript II SK (+) (Stratagene). We termed one of the cDNAs mouse Brorin. Human Brorin cDNA was also identified in a homology-based search of human cDNA sequences in nucleotide sequence databases with the amino acid sequence of mouse Brorin.

Forced Expression of Mouse Brorin cDNA in COS-7 Cells—The mouse Brorin cDNA with a DNA fragment encoding a Myc tag (EQKLISEEDL) and a His 6 tag (HHHHHH) at the 3’ termi-
of the coding region was constructed between EcoRI and XbaI sites in a vector DNA, pcDNA3.1(+) (Invitrogen).

COS-7 cells were plated at ~70–80% confluence (~2 × 10⁴ cells/cm²) in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS) (JRH Biosciences) on 12-well plates and were transfected with the recombinant vector using Lipofectamine 2000 (Invitrogen) at 37 °C for 16 h in a humidified atmosphere of 5% CO₂ in air. After the transfection, COS-7 cells were further cultured in fresh culture medium at 37 °C for 72 h.

Detection of Recombinant Mouse Brorin Protein by Western Blotting—The culture medium and lysate of the transfected cells were separated by SDS-polyacrylamide gel (12.5%) electrophoresis under reducing conditions and transferred onto a nitrocellulose membrane (Hybond-ECL; Amersham Biosciences). The protein with the Myc tag on the membrane was detected using mouse monoclonal anti-Myc tag antibody (Cell Signaling) (1:500) as primary antibody in phosphate-buffered saline (PBS) containing 0.1% Tween 20 and horseradish peroxidase-conjugated rabbit anti-mouse IgG antibody (ZYMED) (1:1,000) as secondary antibody in PBS containing 0.1% Tween 20. Immunoreactive bands were visualized using an enhanced chemiluminescence detection system (PerkinElmer Life Sciences) as described (12). Prestained Protein Marker Broad Range (New England Biolabs) was used as molecular mass standard proteins.

Production of Recombinant Mouse Brorin in Insect Cells—The coding region (without the amino-terminal secreted signal sequence of 27 amino acids) of mouse Brorin cDNA with a DNA fragment encoding a Myc tag and a His₆ tag at the 3’ terminus of the coding region was constructed in a transfer vector DNA, pAcGP67A. Recombinant baculovirus containing the cDNA was obtained by cotransfection of Sf9 cells with the recombinant pAcGP67A and a Bsu36I-digested expression vector, BacPak6.

High Five cells (~2 × 10⁶ cells/ml) infected with the recombinant baculovirus were cultured at 27 °C for 72 h in serum-free medium EX-CELL 400 (JRH Bioscience). Recombinant mouse Brorin was purified from the culture medium by affinity chromatography using Ni-NTA agarose and desalted by gel filtration chromatography using Bio-Gel P-6 DG in PBS containing 100 µg/ml of bovine serum albumin as a carrier. Purified recombinant mouse Brorin was separated by SDS-polyacrylamide gel (12.5%) electrophoresis under reducing conditions followed by protein staining with Coomassie Brilliant Blue R-250. Purified recombinant mouse Brorin was also analyzed by Western blotting as described above.

Reverse Transcription-PCR—RNA was extracted from mouse tissue using an RNeasy Mini kit (Qiagen). Mouse cDNA was transcribed from the RNA as a template with Moloney murine leukemia virus reverse transcriptase. Brorin cDNA was amplified from the cDNA with Tag DNA polymerase and primers specific for mouse Brorin (5’-acgtggttagggagctcgtc-3’, 5’-gtctactttgctgtcactcag-3’). Gapdh cDNA was amplified with primers specific for mouse Gapdh (5’-tgaccagtctccgctct-3’, 5’-ttccacacacctgtgta-3’) as a control. The amplified DNA was analyzed by agarose gel (1.5%) electrophoresis. Thereafter, the gel was stained with ethidium bromide. The expected sizes of Brorin cDNA and Gapdh cDNA are 988 and 454 base pairs, respectively.

In Situ Hybridization—For in situ hybridization, embryos and mouse brain (postnatal day 56, P56) were frozen in powdered dry ice, and sections were cut at 16 µm with a cryostat, thaw-mounted onto poly-L-lysine-coated slides, and stored at −85 °C prior to use. A 35S-labeled mouse sense or antisense Brorin RNA probe was transcribed from SpeI or HindIII-digested mouse brorin cDNA cloned in pBlueScript II SK (+) (Stratagene) using T7 or T3 RNA polymerase with uridine 5’-[α-35S]thiotriphosphate (~30 TBq/mmol) (Amersham Biosciences), respectively. The sections were examined by in situ hybridization with the labeled probe, followed by exposure to x-ray film (BioMax MR; Kodak) for 10 days or dipping in liquid emulsion (Kodak NTB3) diluted 1:1 and exposure for 3 weeks as described (13). The sections of mouse embryos and brain were counterstained with hematoxylin-eosin and cresyl-violet (Nissl staining), respectively. Silver grains were visualized by microscopy.

Alkaline Phosphatase Activity in MC3T3-E1 Cells—Mouse preosteoblastic MC3T3-E1 cells were maintained and subcultured at 37 °C for 3 or 4 days in a humidified atmosphere of 5% CO₂ in air supplemented with 10% FBS, 100 units/ml of penicillin G, and 100 µg/ml of streptomycin in a humidified CO₂ incubator.

For the determination of alkaline phosphatase activity, MC3T3-E1 cells were plated at a density of 1 × 10⁵ cells/well in 48-well plates for 48 h. Once confluent, the cells were cultured in α-MEM containing 10% FBS, 100 units/ml of penicillin G, 100 µg/ml of streptomycin, 10 mM β-glycerophosphate, and 50 µg/ml of ascorbic acid for 72 h. The cells were then cultured in α-MEM containing 0.3% FBS, 100 units/ml of penicillin G, and 100 µg/ml of streptomycin for 24 h and further cultured in α-MEM containing 0.3% FBS, 100 units/ml of penicillin G, 100 µg/ml of streptomycin, recombinant human BMP2 or BMP6 (0 or 10 ng/ml) (R&D Systems), recombinant mouse Noggin (0 or 100 ng/ml) (R&D systems), and recombinant mouse Brorin protein (0–100 ng/ml) for 72 h. The cells were washed twice with ice-cold PBS and scraped in 10 mM Tris-Cl containing 2 mM MgCl₂ and 0.05% Triton X-100, pH 8.2. The cell suspensions were sonicated on ice. Aliquots of supernatants were assayed for protein concentration and alkaline phosphatase activity as described (14). For the alkaline phosphatase activity, the assay mixture contained 10 mM p-nitrophenyl phosphate in 0.1 M sodium carbonate buffer, pH 10, supplemented with 1 mM MgCl₂, and was incubated at 37 °C for 30 min. After 0.1 M NaOH was added, the amount of p-nitrophenol liberated was measured by using a spectrophotometer.

Phosphorylation of Smad Protein in MC3T3-E1 Cells—MC3T3-E1 cells were plated in α-MEM containing 10% FBS, 100 units/ml of penicillin G, and 100 µg/ml of streptomycin at a density of 1 × 10⁵ cells/well in 12-well plates for 48 h. Once confluent, the cells were cultured in α-MEM containing 0.3% FBS, 100 units/ml of penicillin G, and 100 µg/ml of streptomycin for 48 h. They were then cultured in α-MEM containing 0.3% FBS, 100 units/ml of penicillin G, and 100 µg/ml of streptomycin for 24 h and further cultured in α-MEM containing 0.3% FBS, 100 units/ml of penicillin G, 100 µg/ml of streptomycin,
**RESULTS**

**Identification of Mouse and Human Brorin**—We identified mouse cDNAs encoding novel putative secreted proteins by randomly analyzing amino acid sequences predicted from mouse cDNAs of unknown function in the GenBank™ nucleotide sequence data base with a computer program for the prediction of protein localization sites in cells (PSORT). The full-length cDNAs were isolated by PCR with mouse embryonic cDNA as a template. One of them encodes a putative secreted protein of 324 amino acids with a putative signal sequence (27 amino acids) at its amino terminus (GenBank™ accession code AB292670) (Fig. 1A). The protein has two cysteine-rich domains in its core region (Fig. 1, A and B). Positions of 10 cysteine residues in the domains of Brorin are conserved in both the Chordin family proteins and human Brorin.

**Culture of Mouse Neural Precursor Cells**—Neural precursor cells were cultured at 13.5 days in vitro (dissociated neural precursor cells) in a cocktail of neurotropic agents and fibronectin-coated 24-well plates. The cells were further cultured under the various conditions indicated. For immunostaining, cells were fixed with 4% paraformaldehyde in PBS at 30 min at 4°C and washed in PBS at room temperature for 30 min three times. They were then permeabilized with 0.1% Triton X-100 in PBS at room temperature for 30 min and immunostained using primary antibodies in PBS containing 5% bovine serum albumin and 0.1% Triton X-100 and secondary antibodies in PBS. The cells were treated with primary antibodies at 4°C overnight. After three washes for 5 min in PBS, the cells were further treated with the secondary antibodies at room temperature for 1 h. Primary antibodies used were as follows: an anti-microtubule-associated protein 2 (MAP2) mouse monoclonal antibody (1:400) (Sigma) and an anti-glial fibrillary acidic protein (GFAP) rabbit polyclonal antibody (1:100) (Sigma). Secondary antibodies used were rhodamine red- or fluorescein isothiocyanate-conjugated anti-rabbit or mouse IgG antibodies (Sigma) (1:200). Cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Roche Applied Science). The stained cells were observed under a fluorescence microscope. Results are the means ± S.E. for six different fields (at least 200 cells/field) from four independent slides.

**Eagle's F12**—Cells were further cultured under the various conditions indicated. For immunostaining, cells were fixed with 4% paraformaldehyde in PBS at 30 min at 4°C and washed in PBS at room temperature for 30 min three times. They were then permeabilized with 0.1% Triton X-100 in PBS at room temperature for 30 min three times. They were then further treated with the secondary antibodies at room temperature for 1 h. Primary antibodies used were as follows: an anti-microtubule-associated protein 2 (MAP2) mouse monoclonal antibody (1:400) (Sigma) and an anti-glial fibrillary acidic protein (GFAP) rabbit polyclonal antibody (1:100) (Sigma). Secondary antibodies used were rhodamine red- or fluorescein isothiocyanate-conjugated antirabbit or mouse IgG antibodies (Sigma) (1:200). Cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Roche Applied Science). The stained cells were observed under a fluorescence microscope. Results are the means ± S.E. for six different fields (at least 200 cells/field) from four independent slides.
Human Brorin cDNA (accession number AB292671) was also identified in a homology-based search of human cDNA sequences in GenBank™ with the amino acid sequence of mouse Brorin. The amino acid sequence of human Brorin (325 amino acids) was highly similar (~90% identity) to that of mouse Brorin (Fig. 1A). We examined the chromosomal localization of the human Brorin gene by searching the Ensembl database with the nucleotide sequence of human Brorin cDNA. The gene was mapped to chromosome 7 at q12.2.

Forced Expression of Mouse Brorin cDNA in Cultured Cells, COS-7 Cells—To examine whether Brorin is a secreted protein, mouse cDNA encoding Brorin with Myc and His6 tags was expressed in cultured cells, COS-7 cells. Both the culture medium and cell lysate were examined by reducing SDS-polyacrylamide gel electrophoresis followed by Western blotting with anti-Myc tag antibody. No bands were detected in the cell lysate or culture medium of COS-7 cells transfected with the empty vector as a control (data not shown). A major band of ~49 kDa was mainly detected in the culture medium of COS-7 cells transfected with the Brorin expression vector, indicating that Brorin is a secreted protein (Fig. 2A). The observed molecular mass was larger than the calculated molecular mass of the recombinant Brorin protein (~38.1 kDa), indicating that Brorin protein might be subjected to post-translational modification.

Production of Purified Recombinant Mouse Brorin Protein—To prepare purified recombinant mouse brorin protein, mouse Brorin cDNA was expressed in cultured High Five insect cells by infection with a recombinant baculovirus containing the mouse Brorin cDNA with a 3′-terminal extension encoding Myc and His6 tags. Recombinant mouse Brorin protein was purified from the culture medium of High Five cells by affinity chromatography using Ni-NTA agarose. Purified recombinant mouse Brorin was separated by SDS-polyacrylamide gel electrophoresis under reducing conditions followed by protein staining with Coomassie Brilliant Blue R-250 (CBB) and by Western blotting with anti-Myc tag antibody (Western). Prestained protein markers were used as molecular mass standard proteins of 62.0, 47.5, and 32.5 kDa.

Purified recombinant Brorin showed a single band of 48 kDa with a band of bovine serum albumin (66 kDa) as a carrier (Fig. 2B). Purified recombinant mouse Brorin was also analyzed by Western blotting. Purified recombinant Brorin also showed a single band of 48 kDa (Fig. 2B).

Expression of Brorin in Mouse Tissues and Embryos Examined by RT-PCR—The expression of Brorin in mouse tissues (P56) was examined by RT-PCR. Although the expression of Gapdh as a control was detected in all the tissues examined, the expression of Brorin was mostly detected in the brain among major tissues examined, indicating that Brorin was predominantly expressed in the brain (Fig. 3A). We also examined the expression of Brorin in the brain at different developmental stages (E12.5-P56) by RT-PCR. The expression was detected in the brain at all the stages examined. However, the expression gradually increased with development (Fig. 3B).

Expression of Brorin in Mouse Embryos and Adult Brain Examined by in Situ Hybridization—The expression of Brorin in mouse embryos at E12.5, E16.5, and E18.5 was examined by in situ hybridization with a 35S-labeled antisense Brorin cRNA probe using sagittal sections. The expression in embryos was shown by red grains (Fig. 4A). In contrast, essentially no grains were detected in embryos with a 35S-labeled sense Brorin cRNA probe as a control (data not shown). The expression of Brorin was predominantly detected in the developing diencephalon at E12.5 and in the developing neural tissues and tongue at E16.5 and E18.5. However, essentially no Brorin expression was observed in the developing cerebral cortex at E16.5 and E18.5.

The expression of Brorin in the brain at P56 was also examined by in situ hybridization with the antisense Brorin probe using consecutive coronal sections. The expression of Brorin, shown by red grains, was widely detected in the brain (Fig. 4B,
However, prominent expression of Brorin was observed in the diencephalon and medulla oblongata. In contrast, essentially no grains were detected with the sense Brorin cRNA probe as a control (Fig. 4B, j–r).

The cellular distribution of Brorin mRNA in the brain was examined using in situ hybridization followed by microautoradiography. With Nissl staining of brain sections, glial cells appeared as small intensely stained (dark) cells. In contrast, neurons were generally larger and less intensely stained (lighter) because of their volume (17). In the diencephalon and medulla oblongata, Brorin mRNA was detected in neurons, but not in glial cells (Fig. 4C). In other regions of the brain, Brorin mRNA was also detected in neurons (data not shown). These results indicated that Brorin in the brain was expressed in neurons but not glial cells.

Effects of Brorin on BMP Signaling in Cultured Osteoblasts, MC3T3-E1—Positions of 10 cysteine residues in the cysteine-rich domains of Brorin are similar to those in the cysteine-rich domains of members of the chordin family. Most members of this family are regulators of BMP signaling, indicating that Brorin might be also. BMPs were originally identified as proteins in bone that induce ectopic bone and cartilage to form in vivo. BMPs are signaling molecules for the stimulation of osteoblastic differentiation (18). Most are members of the dpp and 60A subgroups of the TGFβ superfamily (4). We examined the effect of recombinant mouse Brorin 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, a marker for osteoblastic differentiation (19). We also examined the effect of recombinant mouse Noggin proteins as a control of a BMP antagonist.

BMP2 and BMP6 greatly stimulated the alkaline phosphatase activity of MC3T3-E1 cells. However, the addition of Brorin significantly reduced the activity of BMP2 and BMP6.

![FIGURE 4. Localization of Brorin mRNA in mouse embryos and adult brain. A, sagittal sections of mouse embryos at E12.5, E16.5, and E18.5 were examined by in situ hybridization with a 35S-labeled antisense mouse Brorin RNA probe. The sections were counterstained with hematoxylin-eosin. Red grains superimposed upon a hematoxylin-eosin stain show the localization of Brorin mRNA. B, coronal sections of mouse brain (P56) were also examined by in situ hybridization with a 35S-labeled antisense (a–i) or sense (j–r) mouse Brorin RNA probe, followed by exposure to x-ray film. Red grains show the location of Brorin mRNA. Scale bar, 5 mm. C, the cellular localization of Brorin mRNA in the diencephalon (a) and medulla oblongata (b) of mouse brain (P56) was examined by in situ hybridization as described above. The sections of the brain were counterstained with cresyl-violet. Black grains in bright field photographs show the location of Brorin mRNA. The arrows and arrowheads indicate neurons and glial cells, respectively. Scale bar, 50 μm.]
activity in MC3T3-E1 cells (Fig. 5A). The activity stimulated by BMP2 and BMP6 was inhibited by Brorin in a dose-dependent manner as well as by Noggin (Fig. 5A), indicating that Brorin is a BMP antagonist.

BMPs also induce the phosphorylation of Smad in MC3T3-E1 cells. To confirm the antagonistic activity of Brorin, we also examined effects of Brorin and Noggin as a control on the phosphorylation of Smad induced by BMPs in MC3T3-E1 cells. BMP2 and BMP6 induced the phosphorylation of Smad1/5/8 in MC3T3-E1 cells (Fig. 5B). The phosphorylation induced by BMP2 and BMP6 was inhibited by Brorin as well as Noggin (Fig. 5B), also indicating that Brorin is a BMP antagonist.

Effects of Brorin on Neuronal and Astrocytic Differentiation in Cultured Neural Precursor Cells—The temporal and regional expression profiles of Brorin indicated its potential roles in neural differentiation. As the prominent expression of Brorin was observed in mouse embryonic diencephalons, we examined the effect of recombinant mouse Brorin protein on the neural differentiation of mouse diencephalic neural precursor cells. As FBS promotes neural differentiation in neural precursor cells (20), we also examined the effect of FBS (10%) on the differentiation as a positive control. After the neural precursor cells had been cultured in the presence of FBS or Brorin protein for 3 days, the differentiation was examined by double immunostaining using anti-MAP2 and GFAP antibodies for neurons and astrocytes, respectively (Fig. 6A). Green and red signals indicate MAP2-positive and GFAP-positive cells, respectively. Blue signals indicate cell nuclei counterstained with DAPI. The effects of FBS and Brorin on the total number of cells and neural differentiation were quantified by counting DAPI-positive cell nuclei and MAP2-positive and GFAP-positive cells (Fig. 6, B–D). FBS significantly increased the total number of cells. FBS also significantly increased the proportion of MAP2-positive cells and generated GFAP-positive cells among the neural precursor cells. In addition, FBS greatly stimulated the outgrowth of neurites in MAP2-positive cells. As Brorin slightly increased the total number of cells, it may promote the proliferation and/or survival of the neural precursor cells. In addition, Brorin increased the proportion of MAP2-positive cells among the neural precursor cells and significantly stimulated their neurite outgrowth. However, treatment with Brorin did not produce GFAP-positive cells. These results indicate that Brorin induced neurogenesis, but not astrogenesis.

DISCUSSION

Brorin Is a Novel Secreted BMP Antagonist of the Chordin Family with a Unique Structure—BMPs play important roles in many organogenic processes, although they were originally identified as factors promoting the ectopic formation of cartilage and bone. The activity of BMP is controlled in part by secreted BMP regulators, including Noggin, members of the Chordin family, and members of the Dan/Cerberus family (3). The Chordin family is a group of proteins with cysteine-rich domains that consist of 10 cysteine residues. The family comprises Chordin, Chordin-like (CHL)/Neuralin, CHL2 (11), Neurogenesin-1 (21, 22), Crossoverless-2 (16, 23), and Kielin/chordin-like protein (24). These members have different num-
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Brorin is a unique member of the Chordin family. Unlike other members, it has only two cysteine-rich domains and no structural similarity to other members of the family. These results indicate that Brorin is quite distinct from that of any other member of the Chordin family, indicating that Brorin might play unique roles in developing and adult neural tissues.

Possible Roles of Brorin in the Developing and Adult Neural Tissues—The developing neural tissues express multiple BMPs, including BMPs 2, 4, 5, 6, and 7. BMPs are also expressed in the postnatal and adult brain. Transcript levels for BMPs generally peak during the perinatal period and then decline in the adult neural tissues (4). However, strong signals are still detectable in discrete structures of the mature brain. For example, the adult hippocampus expresses relatively high levels of BMP5 and BMP6; the cortex: BMP5 and BMP6; the cerebellum: BMP5; the striatum: BMP5 and BMP7; and the brainstem: BMP5 and BMP6 (7, 29, 30). In addition, BMP 2, 4, 5, and 6 are expressed in the adult cortex, hippocampus, striatum, and substantia nigra (10). These expression profiles indicate that BMP signaling plays roles in developing and adult neural tissues.

BMP2, BMP4, and BMP7 inhibit neurogenesis and concomitantly induce astrogenesis in mouse neural precursor cells (5, 6). In contrast to BMPs, Brorin significantly promoted neurogenesis, but not astrogenesis, in mouse neural precursor cells. As Brorin is a BMP antagonist, the present finding is consistent with the findings that BMPs inhibit neurogenesis (5, 6). Recently, we also identified brorin in zebrafish. Zebrafish brorin was also predominantly expressed in the neural tissues in embryos. We generated brorin knockdown zebrafish embryos. Our preliminary analysis of the phenotype shows that development of the brain was greatly impaired, indicating that brorin potentially plays a role in the embryonic development of the brain. Although its physiological role remains to be elucidated, Brorin is expected to act as a BMP antagonist during neurogen-

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esis in both the adult brain and developing neural tissues. The present findings should provide new insights into the roles of BMP signaling in neural tissues.

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