doi:10.1006/cyto.2002.1006, available online at http://www.idealibrary.com on IDEAL®

CARDIOTROPHIN-LIKE CYTOKINE INDUCES ASTROCYTE DIFFERENTIATION OF FETAL NEUROEPITHELIAL CELLS VIA ACTIVATION OF STAT3

Atsumi Uemura,[1,2] Takumi Takizawa,[1,3] Wataru Ochiai,[1] Makoto Yanagisawa,[1] Kinichi Nakashima,[1] Tetsuya Taga[1]*

Cardiotrophin-like cytokine (CLC), also known as novel neurotrophin-1/B cell stimulating factor-3 (NNT-1/BSF-3), is a recently identified member of the interleukin (IL)-6 family of cytokines that share gp130 as a signal-transducing receptor component. In this study, we demonstrate that CLC is expressed in fetal mouse neuroepithelial cells and has a potential to induce their astrocyte differentiation in a synergistic manner with bone-morphogenetic protein (BMP)-2, which is also expressed in the fetal mouse brain. CLC-stimulation led to promoter activation of the gene for an astrocyte marker, glial fibrillary acidic protein (GFAP), which was clearly inhibited by expression of a dominant negative form of a transcription factor, STAT3, or by introduction of a mutation in a single STAT3-binding site in the promoter, suggesting a critical role of STAT3 in the CLC-induced GFAP transcription.

These results suggest that CLC plays a role in astrocyte differentiation via STAT3 activation within the developing brain.

CLC is a newly identified IL-6-related cytokine with highest homology to cardiotrophin-1 (CT-1) and ciliary neurotrophic factor (CNTF).[1] CLC was found initially to support survival of chicken embryonic motor and sympathetic neurons and induce B cell hyperplasia,[2] but its function in cell fate determination of neural precursors has yet to be elucidated. We and others previously reported that IL-6 and related cytokines, IL-11, leukemia inhibitory factor (LIF), CNTF, oncostatin M (OSM) and CT-1 are capable of inducing astrocyte differentiation from fetal mouse neuroepithelial cells[3–10] that contain precursor cells which give rise to both neurons and glial cells including astrocytes.[11–13] IL-6 family cytokines share gp130 as a critical signal transducer in their respective receptor complexes.[14] Upon stimulation with IL-6 family cytokines, gp130 becomes dimerized either with itself or with another dimer partner like LIF receptor (LIFR) or OSM receptor (OSMR),[14–18] depending on the type of cytokines. For instance, the binding of CNTF to its specific receptor (CNTFR) leads to formation of a tripartite receptor complex comprising CNTFR and a gp130/LIFR heterodimer.[19–21] The dimerization of gp130 triggers the activation of associating cytoplasmic tyrosine kinases in the Janus kinase (JAK) family and then a downstream transcription factor, STAT3 (for signal transducer and activator of transcription 3). CLC has been suggested to associate with cytokine like factor-1 (CLF) to form a functional heteromeric ligand for the tripartite CNTF receptor complex.[22–24] This CLC/CLF composite cytokine induces gp130/LIFR heterodimerization via CNTFR, and activates JAK and STAT3 like other member of IL-6 family cytokines.

BMPs are members of the transforming growth factor (TGF)-β superfamily and signal through heterotetrameric serine/threonine kinase receptors.[25–27] Activated BMP receptors phosphorylate carboxy-terminally located serine residues in the downstream transcription factors Smad1, -5 or -8, which allows them to associate with a common mediator Smad4, leading to up-regulation of the expression of specific

From the [1]Department of Cell Fate Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan; [2]Department of Pediatrics, Kumamoto University School of Medicine, Kumamoto, Japan; [3]Department of Pediatrics, School of Medicine, Gunma University, Gunma, Japan

Correspondence to: Tetsuya Taga, Ph.D., Professor, Department of Cell Fate Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1, Honjo, Kumamoto, 860-0811, Japan. E-mail, taga@kaiju.medic.kumamoto-u.ac.jp

1043–4666/02/$-see front matter

KEY WORDS: astrocyte/BMP2/cardiotrophin-like cytokine/gp130/STAT3

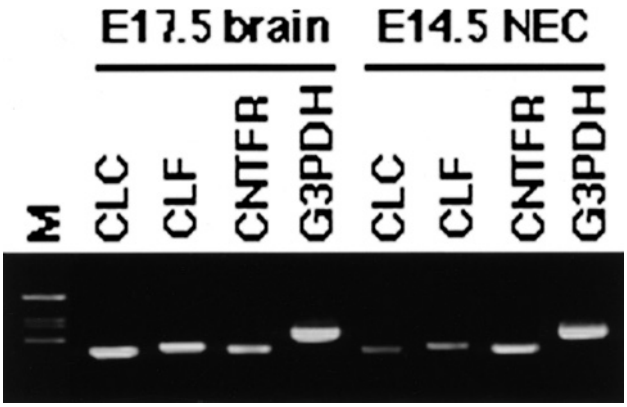

Figure 1. Expression of CLC and CLF transcripts in fetal brain and neuroepithelial cells.

Total RNA from E17.5 mouse whole brain or E14.5 neuroepithelial cells (NEC) cultured for 4 days was subjected to RT-PCR analysis for CLC, CLF, CNTFR and G3PDH. M indicates molecular marker.

genes.[25–27] We have previously reported that LIF and BMP2 synergistically induce astrocyte differentiation of neuroepithelial cells.[4,6] In the current report, we demonstrate that CLC is also capable of cooperating with BMP2 to promote astrocyte differentiation from neuroepithelial cells in a gp130 dependent manner via activation of STAT3.

RESULTS

Expression of transcripts for CLC and CLF in fetal brain and cultured neuroepithelial cells

We have previously observed the expression of IL-6, IL-11, LIF, CT-1, OSM and their receptor components in fetal mouse brain and primary-cultured neuroepithelial cells.[3,7–10] Furthermore, all the IL-6 family cytokines, except for a newly identified CLC, have been shown to induce astrocyte differentiation from neuroepithelial cells via stimulation of shared-signal transducing receptor component, gp130.[3–8,10,12] It has been suggested that these gp130-stimulating cytokines contribute to astrocytogenesis in the developing brain, and CLC may do the same. In the present study, we investigated the expression of CLC in fetal brain as an initial step to examine possible involvement of CLC in astrocytogenesis in vivo. As shown in Figure 1, CLC transcript was clearly detectable in mouse brain on embryonic day (E) 17.5, the time when astrocytes are considered to emerge in vivo. Transcripts for CLF, the heterodimeric partner of CLC, and CNTFR were also observed in the fetal brain. The expression of these transcripts was similarly observed in the cultured mouse neuroepithelial cells prepared from E14.5 brain. These results implied that CLC may participate in astrocytogenesis in the brain during development.

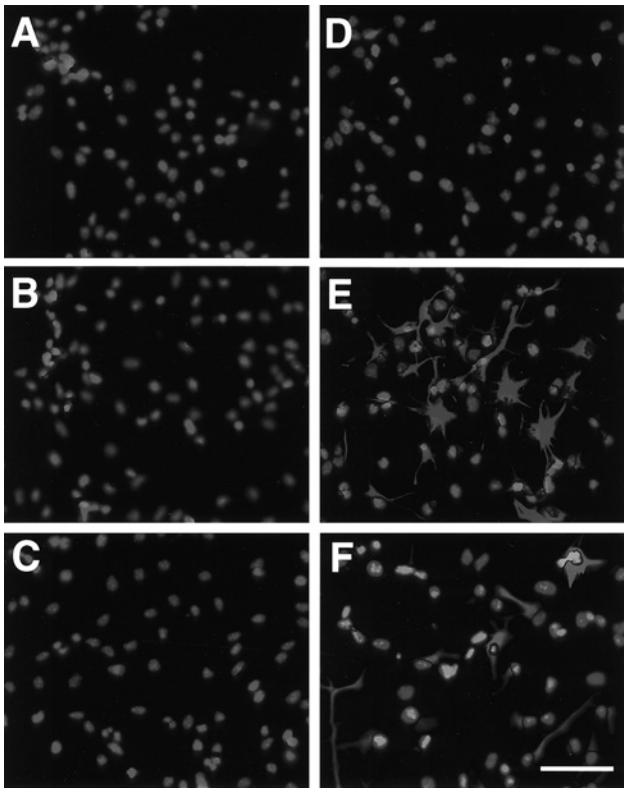

Figure 2. Synergistic astrocyte differentiation induced by CLC and BMP2.

Neuroepithelial cells were cultured with medium alone (A), LIF (80 ng/ml) (B), CLC (800 ng/ml) (C), BMP2 (80 ng/ml) (D), BMP2 (80 ng/ml) plus LIF (80 ng/ml) (E), or BMP2 (80 ng/ml) plus CLC (800 ng/ml) (F) for 2 days. Cells were stained for GFAP (red) and DNA (blue) and observed by fluorescent microscopy. Scale bar, 50 μm.

Synergistic astrocyte differentiation induced by CLC and BMP2

Mouse telencephalic neuroepithelial cells are known to contain neural precursors which give rise to neurons, astrocytes, and oligodendrocytes.[28,29] We have previously observed that any combination of gp130-stimulating cytokines and BMPs which we examined so far, e.g. LIF together with BMP4, LIF together with BMP7, or CT-1 together with BMP2, induces astrocyte differentiation of neuroepithelial cells in a synergistic manner.[4,6,10,30] Thus, we examined whether CLC has a potential to induce astrocytes from neuroepithelial cells in cooperation with BMP2. To monitor the differentiation of astrocytes, we performed immunofluorescent staining with an antibody to an astrocytic marker, glial fibrillary acidic protein (GFAP). As shown in Figure 2, when the neuroepithelial cells were treated with CLC, LIF or BMP2 alone for 2 days, no GFAP-positive astrocytes were observed. On the contrary, GFAP-positive cells with

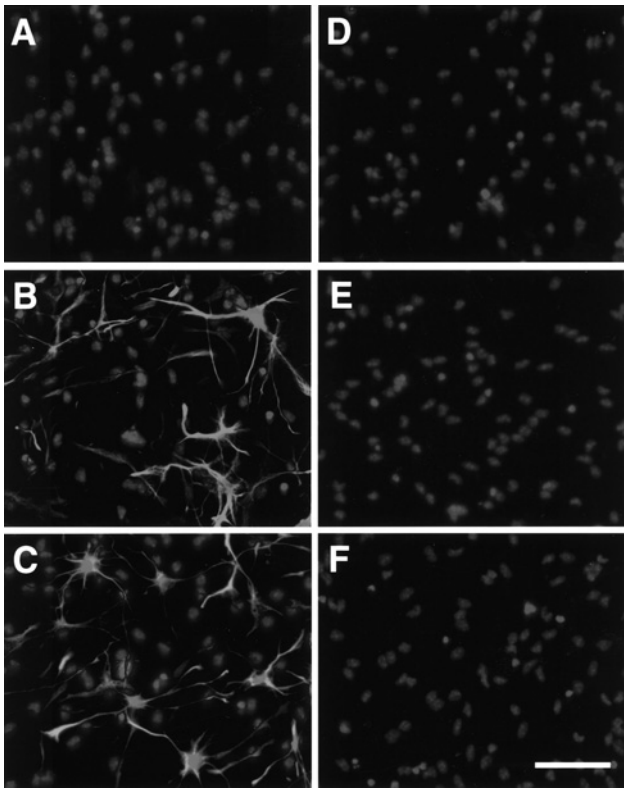

Figure 3. Requirement of gp130 signallings in CLC-induced astrocyte differentiation.

Neuroepithelial cells from E14.5 normal (A, B, C) and gp130–deficient (D, E, F) littermates were cultured with medium alone (A, D), LIF (80 ng/ml) (B, E) or CLC (800 ng/ml) (C, F) for 4 days. Cells were stained for GFAP (stellate-shaped cells) and DNA (small dots), and observed by fluorescent microscopy. Scale bar, 50 μm.

astrocyte-like morphology were clearly detected in the culture with a combination of CLC and BMP2 or LIF and BMP2, indicating a potential of CLC to induce astrocyte differentiation in cooperation with BMP2. Since we added CLC without CLF in these culture, relatively higher concentration of CLC, i.e. 800 ng/ml, was required to exhibit astrocytogenesis to an extent similar to that observed with 80 ng/ml LIF (see discussion for the detail).

CLC-induced astrocyte differentiation of neuroepithelial cells in culture for a longer period

We have previously observed that E14.5 mouse embryonic neuroepithelial cells differentiate into astrocytes in the presence of previously identified IL-6 family cytokines alone without the addition of BMP2, when cultured for a longer period (4 or 6 days).[4,5,7,8,10] We thus examined whether CLC could induce astrocytes in a 4-day culture of the neuroepithelial cells from E14.5 mouse telencephalon. As shown in Figure 3B and C, CLC did actually induce GFAP-positive astrocytes in cultured neuroepithlial cells to the extent comparable to that observed with LIF. When neuroepithlial cells from gp130-deficient littermates were used for the same assay, neither of these gp130-stimulating cytokines induced astrocyte differentiation due to the requirement of gp130 for their signal transduction (Figure 3 D, E, F).

CLC-induced tyrosine phosphorylation of gp130, LIFR and STAT3 in neuroepithelial cells

It has been shown that tyrosine-phosphorylation of gp130, LIFR and a gp130-downstream signalling molecule, STAT3, rapidly occurs upon stimulation of cells with CNTF.[16,19,20,24] Based on the results reported previously that CLC signals through the tripartite CNTFR complex comprising CNTFR, gp130 and LIFR,[24] we examined whether CLC actually induces the phosphorylation of gp130, LIFR and STAT3 in neuroepithelial cells. As shown in Figure 4, gp130 and STAT3 were clearly phosphorylated on tyrosine residue in response to CLC stimulation. It should be noted that LIFR which was co-immunoprecipitated with gp130 (see ref. 9) was also tyrosine-phosphorylated (Figure 4, top panel). Contrary to these results, no such tyrosine-phosphorylation was observed in cells deficient for gp130, confirming the indispensableness of gp130 for CLC to signal.

STAT3-mediated activation of the GFAP gene promoter by CLC

The phosphorylation on tyrosine^{705} in the STAT3 protein in response to gp130-stimulating cytokines has been reported to be critical for STAT3 dimerization and for gp130-mediated signal transduction. Thus, a STAT3 mutant whose tyrosine^{705} is substituted to phenylalanine completely abolishes its signalling capability. Furthermore, this mutated form of STAT3 (STAT3^{Y705F}) functions as a dominant-negative molecule against endogenous STAT3 when overexpressed. To examine the involvement of STAT3 in CLC-induced astrocyte differentiation, we performed luciferase assay using a reporter construct containing GFAP gene promoter (GF1L-pGL3). As shown in Figure 5A, CLC-induced GFAP promoter activation in neuroepithelial cells was dramatically impeded by forced-expression of STAT3^{Y705F}, indicating that STAT3 plays an important role in CLC-induced astrocyte differentiation as assessed by GFAP expression.

STAT3 recognition site in the GFAP promoter critical for CLC-induced activation

We have previously reported that a potential STAT3 binding element (TTCCGAGAA, -1518 to -1510) in the mouse GFAP promoter is important for its activation induced by LIF, CNTF, OSM, IL-11, CT-1 and IL-6.[3–10] To confirm whether this sequence is also important for CLC-induced activation of GFAP

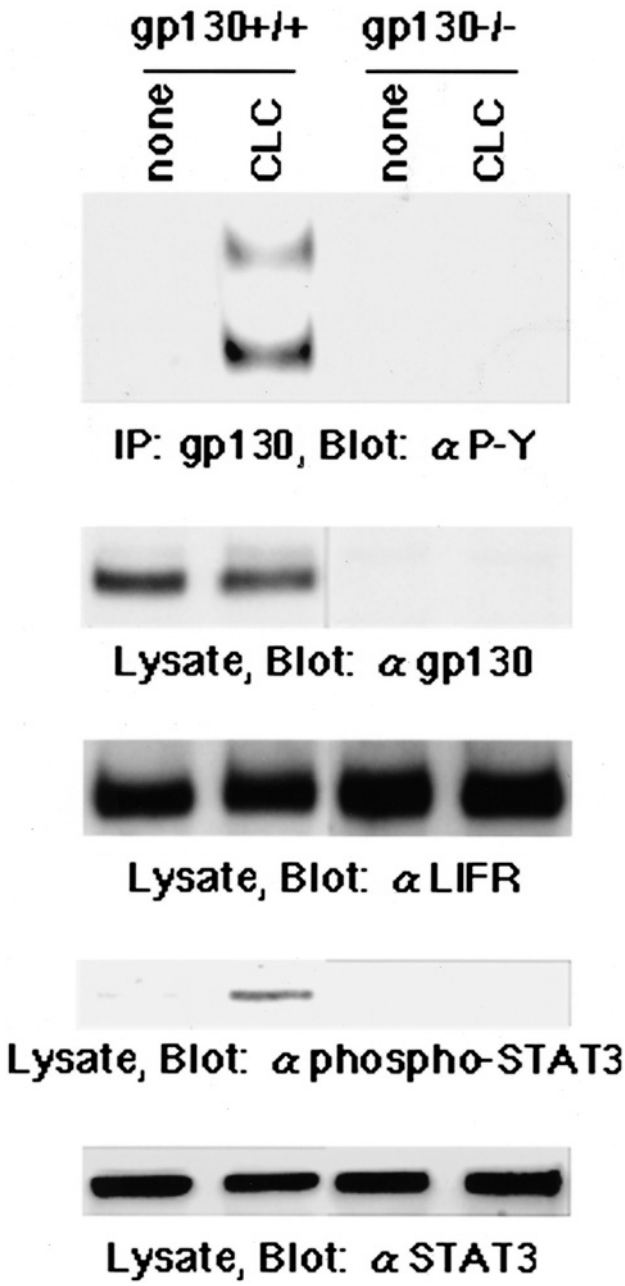

Figure 4. CLC-induced tyrosine-phosphorylation of gp130, LIFR and STAT3.

Neuroepithelial cells prepared from gp130 +/+ or gp130 −/− mice were stimulated with medium alone, CLC or LIF. NP40 lysates from these cells were subjected to immunoprecipitation with antibody to gp130. Precipitates and cell lysates were separated on SDS-PAGE and probed with antibodies to phosphotyrosine, gp130, LIFR, phospho-STAT3 and STAT3.

promoter, we introduced a nucleotide substitution in this STAT3 binding element (TTCCGAGAA to CCAAGAGAA) in the promoter. As shown in Figure 5B, this substitution drastically diminished the responsiveness of the GFAP gene promoter to CLC-stimulation, indicating that the TTCCGAGAA sequence located approximately 1.5 kb upstream of the

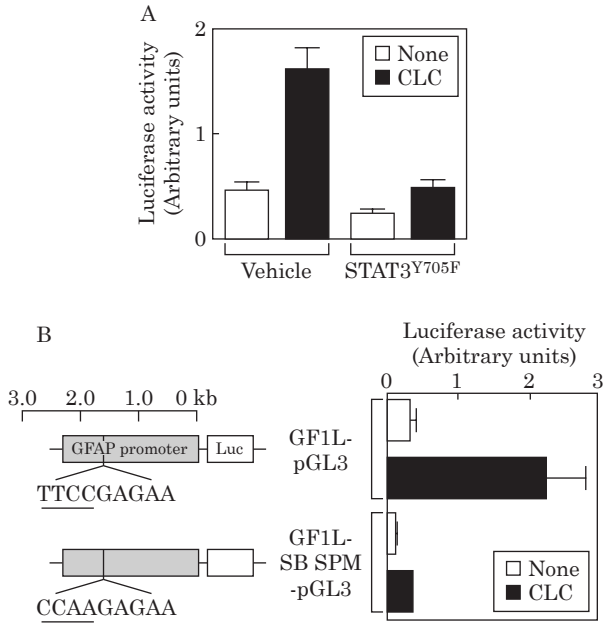

Figure 5. Involvement of STAT3 in CLC-induced GFAP promoter activation.

Neuroepithelial cells were transfected with expression plasmid encoding $STAT3^{Y705F}$ or control vehicle together with GF1L-pGL3 and R-Luc. The cells were stimulated with or without CLC (800 ng/ml) for 8 h (A). Cells in (B) were transfected with either GF1L-pGL3 or GF1L-SBSPM-pGL3 along with R-Luc and treated as described in (A).

transcriptional start site is critical for CLC-induced activation of the gene transcription.

DISCUSSION

In this paper, we have shown that CLC cooperates with BMP2 to induce astrocytes in the 2–day culture of the neuroepithelial cells as has been expected from the previous finding with LIF and BMP2.[4] We have further demonstrated that CLC induces astrocyte differentiation in the 4-day culture of the neuroepithelial cells in which BMP2 is suggested to be expressed and accumulated at a concentration sufficient to synergize with CLC. This action of CLC is dependent on gp130, since no astrocytes come out from the gp130 deficient neuroepithelial cells. We have also shown here that CLC activates GFAP promoter and that the activation is diminished by forced expression of a dominant negative form of STAT3 ($STAT3^{Y705F}$) or mutating a STAT3 responsive element in the promoter, suggesting that STAT3 plays a critical role in CLC-induced astrocyte differentiation.

In the present study, we have found that relatively higher concentration (800 ng/ml) is required for CLC to induce astrocyte differentiation at an extent similar to that induced by LIF (80 ng/ml). The weak activity of CLC seems likely to be attributed to the requirement of

CLF for CLC to form a functional heterodimer to exhibit its function.[22–24] In support of this, the joint addition of CLC at a concentration of 80 ng/ml and conditioned medium of soluble CLF-expressing NIH3T3 cells showed GFAP promoter activation at a level similar to that induced by LIF (80 ng/ml) in neuroepithelial cells (data not shown).

The molecular mechanism for synergistic function between LIF and BMP2 has been explained by the fact that their respective downstream transcription factors, STAT3 and Smad1, form a complex bridged by a transcriptional coactivator p300 to fully activate astrocyte specific gene promoter.[6] Since CLC induces STAT3 activation as LIF does, it is likely that CLC employs the same mechanism to LIF when it acts in cooperation with BMP2 to induce astrocyte differentiation from neuroepithelial cells. Taken together, it is conceivable that all the IL-6 family cytokines, which have a potential to activate STAT3, are capable of collaborating with BMP2 to induce astrocyte differentiation from neuroepithelial cells. We have observed the expression of the IL-6 family cytokines, BMPs (at least BMP2, BMP4 and BMP7) and their receptor components in fetal neuroepithelial cells.[4,7,8,10] Considering our previous results showing that the number of GFAP positive astrocytes in the E18.5 gp130 deficient brain was severely reduced even though the expression of BMPs and their cognate receptors was unaffected,[5] it seems most likely that signals from IL-6 family cytokines and BMPs are neither dispensable for the elaboration of astrocytes in fetal brain during development.

MATERIALS AND METHODS

Animals and Cell culture

Time-pregnant ICR mice were used to prepare neuroepithelial cells. Mice were treated according to the guidelines of Kumamoto University Animal Committee. Neuroepithelial cells were prepared from telencephalons of E14.5 mice and cultured as described previously.[5,6] In brief, the telencephalons were triturated in Hanks' balanced salt solution (HBSS) by mild pipetting with 1 ml pipet tip (Gilson, Middleton, WI, USA). Dissociated cells were cultured for 4 days in N2-supplemented Dulbecco's modified Eagle's medium with F12 containing 10 ng/ml of basic FGF (R&D systems, Minneapolis, MN, USA) (N2/DMEM/F12/bFGF) on 10 cm dish which had been precoated with poly-L-ornithine (Sigma, St. Louis, MO, USA) and fibronectin (Life Technologies, Gaithersburg, MD, USA). Cells were then detached in HBSS and replated on Chamber Slide (Nunc, Naperville, IL, USA) precoated as above, and cultured in the N2/DMEM/F12/bFGF medium supplemented with or without CLC/NNT-1/BSF-3 (provided by Dr Giorgio Senaldi, Amgen Inc.).

RT-PCR

Total RNAs were isolated from 4 day-cultured E14.5 neuroepithelial cells and E17.5 whole brain. cDNAs were synthesized from 5 μg of total RNAs as templates in 20 μl of reaction mixture using Superscript II reverse transcriptase (Gibco BRL, USA). After reverse transcription, each reaction mixture was diluted five-fold with H_2O, and 1 μl of each mixture was subjected to PCR using AmpliTaq Gold DNA polymerase (Applied Biosystems Japan, Tokyo, Japan) for hot start PCR with the following setting: 95°C for 9 min; 40 cycle of 94°C 20 s, 60°C 20 s, and 72°C 30 s ; 72°C 5 min. The following specific primers were synthesized. For CLC, forward primer CAGAACTATGAGGCGTACAGTC (nucleotides 407–428 of the mouse CLC cDNA sequence;[2] GenBank accession number NM019952); reverse primer TGCAGGGTGACTGAAGCTGC (nucleotides 750–731). For CLF, forward primer TACCTGCTCTATACATGGA GAC (nucleotides 304–325 of the mouse CLF cDNA sequence; GenBank accession number NM018827); reverse primer TGACCGTACCACCTCAGCTTGTAC (nucleotides 669–646). For CNTFR, forward primer AACGGC TCTCAGCTGATACTG (nucleotides 363–383 of the mouse CNTFR cDNA sequence; GenBank accession number NM016673); reverse primer GCGTTGCTGACACTTATG GAG (nucleotides 712–692). For glyceraldehyde-3-phosphate dehydrogenase (G3PDH), forward primer ACC ACAGTCCATGCCATCAC; reverse primer TCCACCAC CCTGTTGCTGTA. PCR products were electrophoresed on a 1.2% agarose gel, and results were analysed by EtBr staining.

Immunofluorescent staining

Cells cultured on Chamber Slides were washed with PBS and fixed in 4% paraformaldehyde in PBS and stained with anti-GFAP antibody (Dako, High Wycombe, UK) and rhodamine-conjugated second antibody (Chemicon, Temecula, CA, USA). To stain nuclei, bisbenzimide H33258 fluorochrome trihydrochloride (Nacaraitesque, Kyoto, Japan) was used.

Immunoblotting

Cells stimulated with CLC (800 ng/ml) or LIF (80 ng/ml) were dissolved with NP40 lysis buffer [0.5% NP40, 10 mM Tris-Cl pH 7.4, 150 mM NaCl, 3 mM pAPMSF (Wako Chemicals, Osaka, Japan), 5 μg/ml aprotinine (Sigma), 2 mM sodium orthovanadate (Wako Chemicals), 5 mM EDTA]. Lysates were immunoprecipitated with antibodies to gp130 (Santa Cruz Biotechnology, Santa Cruz, CA). Precipitates and cell lysates were subjected to SDS-PAGE and subsequent immunoblotting with antibodies to phosphotyrosine (4G10; Upstate Biotechnology, Lake Placid, NY, USA), gp130, LIFR (Santa Cruz Biotechnology), phospho-STAT3(Tyr705) (Cell Signaling Technology, Beverly, MA, USA) and STAT3 (Transduction Laboratories, Lexington, KY, USA). Detection was performed with an ECL system (Amersham, Arlington Heights, IL, USA).

GFAP promoter assay

Neuroepithelial cells cultured for 4 days on 10 cm dish as described above were replated on 12-well plate (Nunc) and

transfected on the next day with a plasmid containing firefly luciferase gene under the regulation of 2.5 kb GFAP promoter (GF1L-pGL3) or a modified construct, in which nucleotide substitutions were introduced in the TTCCGA GAA sequence, a potential STAT3-binding site (GF1L-SBSPM-pGL3; see Figure 5B). Control transfection was made with sea pansy luciferase gene conjugated with human elongation factor 1α promoter (R-Luc, kindly provided by Drs Shigekazu Nagata and Koji Shimozaki, Osaka University). Transfection was performed by using Trans-It LT1 (Pan Vera, Madison, WI, USA) according to manufacturer's procedures. On the following day, cells were stimulated with CLC (800 ng/ml) for 8 h and then solubilized and luciferase activity was measured according to the recommended procedures for Pikkagene Dual Luciferase Assay System (Tokyo Ink Inc., Tokyo, Japan) with some modification: Trypsin inhibitor (1 mg/ml, Type III-0 from soy bean white; Sigma) was supplemented in cell lysis buffer. Luminous CT-9000D (Dia-Iatron, Tokyo, Japan) luminometer was used for the detection.

Acknowledgements

We thank Dr Giorgio Senaldi, Amgen Inc. for providing CLC/NNT-1/BSF-3, Yamanouchi Pharmaceutical Co., Ltd. for BMP2, and Drs Shigekazu Nagata and Koji Shimozaki for pEF-Rluc. We are very much grateful to Ms Yuki Noguchi for her excellent secretarial assistance. We also thank Ms Kaori Kaneko for technical help. This work was supported by Grant-in-Aid from Ministry of Education, Culture, Sports, Science and Technology, Takeda Science Foundation, and Human Frontier Science Program. M. Y. is supported by the Research Fellowships of the Japan Society for Promotion of Science for Young Scientists.

References

1. Shi Y, Wang W, Yourey PA, Gohari S, Zukauskas D, Zhang J, Ruben S, Alderson RF (1999) Computational EST database analysis identifies a novel member of the neuropoietic cytokine family. Biochem Biophys Res Commun 262:132–138.

2. Senaldi G, Varnum BC, Sarmiento U, Starnes C, Lile J, Scully S, Guo J, Elliott G, McNinch J, Shaklee CL, Freeman D, Manu F, Simonet WS, Boone T, Chang MS (1999) Novel neurotrophin-1/B cell-stimulating factor-3: a cytokine of the IL-6 family. Proc Natl Acad Sci USA 96:11458–11463.

3. Bonni A, Sun Y, Nadal-Vicens M, Bhatt A, Frank DA, Rozovsky I, Stahl N, Yancopoulos GD, Greenberg ME (1997) Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway. Science 278:477–483.

4. Nakashima K, Yanagisawa M, Arakawa H, Taga T (1999) Astrocyte differentiation mediated by LIF in cooperation with BMP2. FEBS Lett 457:43–46.

5. Nakashima K, Wiese S, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Yoshida K, Kishimoto T, Sendtner M, Taga T (1999) Developmental requirement of gp130 signaling in neuronal survival and astrocyte differentiation. J Neurosci 19: 5429–5434.

6. Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M, Miyazono K, Taga T (1999) Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. Science 284:479–482.

7. Yanagisawa M, Nakashima K, Taga T (1999) STAT3-mediated astrocyte differentiation from mouse fetal neuroepithelial cells by mouse oncostatin M. Neurosci Lett 269:169–172.

8. Yanagisawa M, Nakashima K, Arakawa H, Ikenaka K, Yoshida K, Kishimoto T, Hisatsune T, Taga T (2000) Astrocyte differentiation of fetal neuroepithelial cells by interleukin-11 via activation of a common cytokine signal transducer, gp130, and a transcription factor, STAT3. J Neurochem 74:1498–1504.

9. Takizawa T, Yanagisawa M, Ochiai W, Yasukawa K, Ishiguro T, Nakashima K, Taga T (2001) Directly linked soluble IL-6 receptor-IL-6 fusion protein induces astrocyte differentiation from neuroepithelial cells via activation of STAT3. Cytokine 13: 272–279.

10. Ochiai W, Yanagisawa M, Takizawa T, Nakashima K, Taga T (2001) Astrocyte differentiation of neuroepithelial cells involving cardiotrophin-1-induced activation of STAT3. Cytokine 14:264–271.

11. Reynolds BA, Tetzlaff W, Weiss S (1992) A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. J Neurosci 12:4565–4574.

12. Johe KK, Hazel TG, Muller T, Dugich-Djordjevic MM, McKay RD (1996) Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Genes Dev 10:3129–3140.

13. Rajan P, McKay RD (1998) Multiple routes to astrocytic differentiation in the CNS. J Neurosci 18:3620–3629.

14. Taga T, Kishimoto T (1997) Gp130 and the interleukin-6 family of cytokines. Annu Rev Immunol 15:797–819.

15. Murakami M, Hibi M, Nakagawa N, Nakagawa T, Yasukawa K, Yamanishi K, Taga T, Kishimoto T (1993) IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. Science 260:1808–1810.

16. Davis S, Aldrich TH, Stahl N, Pan L, Taga T, Kishimoto T, Ip NY, Yancopoulos GD (1993) LIFRβ and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. Science 260:1805–1808.

17. Kishimoto T, Taga T, Akira S (1994) Cytokine signal transduction. Cell 76:253–262.

18. Tanaka M, Hara T, Copeland NG, Gilbert DJ, Jenkins NA, Miyajima A (1999) Reconstitution of the functional mouse oncostatin M (OSM) receptor: molecular cloning of the mouse OSM receptor β subunit. Blood 93:804–815.

19. Ip NY, Nye SH, Boulton TG, Davis S, Taga T, Li Y, Birren SJ, Yasukawa K, Kishimoto T, Anderson DJ, Stahl N, Yancopoulos GD (1992) CNTF and LIF act on neuronal cells via shared signaling pathways that involve the IL-6 signal transducing receptor component gp130. Cell 69:1121–1132.

20. Ip NY, McClain J, Barrezueta NX, Aldrich TH, Pan L, Li Y, Wiegand SJ, Friedman B, Davis S, Yancopoulos GD (1993) The α component of the CNTF receptor is required for signaling and defines potential CNTF targets in the adult and during development. Neuron 10:89–102.

21. Davis S, Aldrich TH, Ip NY, Stahl N, Scherer S, Farruggella T, DiStefano PS, Curtis R, Panayotatos N, Gascan H, Chevalier S, Yancopoulos GD (1993) Released form of CNTF receptor α component as a soluble mediator of CNTF responses. Science 259:1736–1739.

22. Elson GC, Lelievre E, Guillet C, Chevalier S, Plun-Favreau H, Froger J, Suard I, de Coignac AB, Delneste Y, Bonnefoy JY, Gauchat JF, Gascan H (2000) CLF associates with CLC to form a functional heteromeric ligand for the CNTF receptor complex. Nat Neurosci 3:867–872.

23. Plun-Favreau H, Elson G, Chabbert M, Froger J, deLapeyriere O, Lelievre E, Guillet C, Hermann J, Gauchat JF, Gascan H, Chevalier S (2001) The ciliary neurotrophic factor receptor α component induces the secretion of and is required for functional responses to cardiotrophin- like cytokine. Embo J 20:1692–1703.

24. Lelievre E, Plun-Favreau H, Chevalier S, Froger J, Guillet C, Elson GC, Gauchat JF, Gascan H (2001) Signaling pathways recruited by the cardiotrophin-like cytokine/cytokine-like factor-1 composite cytokine. Specific requirement of the membrane-bound

form of ciliary neurotrophic factor receptor α component. J Biol Chem 276:22476–22484.

25. Heldin CH, Miyazono K, ten Dijke P (1997) TGF-β signalling from cell membrane to nucleus through SMAD proteins. Nature 390:465–471.

26. Massague J, Chen YG (2000) Controlling TGF-β signaling. Genes Dev 14:627–644.

27. Massague J (2000) How cells read TGF-β signals. Nat Rev Mol Cell Biol 1:169–178.

28. McKay R (1997) Stem cells in the central nervous system. Science 276:66–71.

29. Gage FH (2000) Mammalian neural stem cells. Science 287:1433–1438.

30. Yanagisawa M, Nakashima K, Takizawa T, Ochiai W, Arakawa H, Taga T (2001) Signaling crosstalk underlying synergistic induction of astrocyte differentiation by BMPs and IL-6 family of cytokines. FEBS Lett 489:139–143.