Journal of Neuroimmunology 327 (2019) 36–40

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Short communication

Reduced levels of Coco in sera of multiple sclerosis patients: A potential role in neuro-regeneration failure

Karin Mausner-Fainberg[a], Moran Penn[b], Maya Golan[a], Moshe Benhamou[a,b], A. Wilf-Yarkoni[e], Smadar Gertel[b,c], Arnon Karni[a,b,d,*]

[a] Neuroimmunology Laboratory, Department of Neurology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
[b] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[c] Department of Rheumatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
[d] Segol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel
[e] Department of Neurology, Tel Aviv Sourasky Medical Center, Israel

ARTICLE INFO

Keywords:
Coco
Bone morphogenetic proteins
Multiple sclerosis
Oligodendrocytes precursor cells (OPCs)
Oligodendrogenesis
P19 cells

ABSTRACT

Demyelination, axonal loss and failure of tissue repair characterize MS lesions. Bone morphogenetic proteins (BMPs) signaling is associated with remyelination failure. Coco is one of the BMP antagonists. We found reduced Coco serum levels in relapsing-remitting MS (RR-MS) and primary progressive MS (PP-MS) patients compared to matched healthy controls (HC) and patients with rheumatoid arthritis. Exposure of P19 cells, in the presence of retinoic acid, BMP-2, or BMP-4 to Coco, at average sera level of MS patients failed to induce neuronal phenotype, in contrast to the average sera level of HC. Coco may be a player in the BMP dysregulation and the tissue repair failure in MS.

1. Introduction

Demyelination in the CNS is the major damage in multiple sclerosis (MS). Oligodendrocyte precursor cells (OPCs) are responsible for spontaneous remyelination (Lassmann et al., 1997). OPCs are present in the lesions (Chang et al., 2002; Wolswijk, 1998), but usually they fail to differentiate and to remyelinate, due to the presumably presence of maturation inhibitors (Kuhlmann et al., 2008).

Bone morphogenetic proteins (BMPs) are potent inhibitors of oligodendrocytes differentiation (Gross et al., 1996; Mabie et al., 1997), also they were also been reported to induce quiescence of neural stem cells (Mira et al., 2010). BMPs upregulation was demonstrated in various models of demyelination (Fuller et al., 2007; Ara et al., 2007), and in MS lesions (Deininger et al., 1995).

CNS- infiltrating immune cells may be a source for BMPs in MS lesions (Deininger et al., 1995) as peripheral blood mononuclear cells (PBMCs) of RR-MS patients secrete elevated BMP-2, −4, and − 5 levels (Mausner-Fainberg et al., 2013), concomitantly with reduced levels of BMP antagonists, noggin, and follistatin (Urshansky et al., 2011a, 2011b). In sera of patients with RR-MS, BMP-2 but not BMP-4 levels were found to be high (Penn et al., 2017), and the levels of differential screening-selected gene that is aberrative in neuroblastoma (DAN) which is a BMP antagonist were reduced (Mausner-Fainberg et al., 2016).

Coco, also known as Dand5, Dante, Cer2, Cerl2, and Grem3, is a member of the DAN family of BMP antagonists. Similar to other DAN family members, Coco was found to bind BMPs, specifically BMP4, and to block their interaction with the BMP receptors. (Hsu et al., 1998; Pearce et al., 1999). In human, Coco transcripts were observed in the in brain, heart, adrenal, testes at the embryonic and adult stages (Zhou et al., 2015; Fagerberg et al., 2014).

We tested Coco levels in serum, CSF, and supernatants of cultured PBMCs of RR-MS patients compared to matched healthy controls (HC). The biologic significance of the gap in sera Coco levels between RR-MS patients and HC was examined in a P19 cell model.

2. Methods

2.1. Subjects

Serum was obtained from 41 untreated RR-MS patients, 37 interferon–β1a (IFN-β1a) treated RR-MS patients for at least 3 months,

Abbreviations: BMP, Bone morphogenic protein; CSF, cerebrospinal fluid; DAN, differential screening-selected gene aberrative in neuroblastoma; EAE, experimental autoimmune encephalomyelitis; HC, healthy controls; RRMS, relapsing remitting multiple sclerosis; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis

* Corresponding author at: Department of Neurology, Tel Aviv Sourasky Medical Center, 6 Weizmann Street, Tel Aviv 6423906, Israel.
E-mail address: arnonk@tlvmc.gov.il (A. Karni).

https://doi.org/10.1016/j.jneuroim.2019.01.009
Received 22 October 2018; Received in revised form 4 January 2019; Accepted 16 January 2019

Table 1
Clinical characteristics of the study participants.

	Healthy controls	Untreated RR-MS patients	IFN-β treated RR-MS patients	PP-MS patients	RA patients
Number	36	41	37	15	20
Male	8	6	11		
Female	28	35	26		
Age (mean ± SD)	35.6 ± 7.7	39.7 ± 10.1	45.3 ± 11.4	55.3 ± 9.6	59.4 ± 11.2
Age range, years	25–65	21–62	27–73	39–73	35–75
EDSS (mean ± SD)	–	1.4 ± 1.5	1.1 ± 1.3	5.5. ± 2.2	–
EDSS range	–	0–5.5	0–4	2–8.5	–
Disease duration (mean ± SD)	–	6.8 ± 7.4	13.8 ± 9.5	11.6. ± 9.3	13.3 ± 9.6
Disease duration range, years	–	1–26	1–39	4–40	2–34

15 patients with PP-MS, 36 HC and 20 patients with rheumatoid arthritis (RA) that were served as another immunological disease control. Blood for PBMC purification and CSF samples were drawn from 5 untreated RR-MS patients, 5 IFN-β1a-treated patients, and 5 controls (noninfectious/noninflammatory neurological patients). All RR-MS patients were in clinical remission for at least 3 months and the PP-MS patients were untreated. No patient had other chronic inflammatory condition. The institutional ethics committee approved the experiments, and informed consent was obtained from all participants. Table 1 describes the study participant's characteristics.

2.2. PBMCs isolation and culture

PBMCs were purified by centrifugation over Ficoll–Paque (Amersham Biosciences, Uppsala, Sweden). Cultured PBMCs were either unstimulated or with mouse anti-human CD3 (1 μg/ml) and anti-human CD28 (5 μg/ml) or their isotype controls (R&D System, Minneapolis, MN, USA), or with 100 ng/ml lipopolysaccharides (LPS, Sigma–Aldrich, St. Louis, MO, USA). Supernatants of 24 and 48 h were collected after incubation at 37 °C, 5% CO_2.

2.3. Coco detection by ELISA

Coco levels were measured in sera, CSF, and culture supernatants by ELISA (human Coco DuoSet, R&D Systems). A Termo Max ELISA reader (Molecular Devices microplate reader, USA) used for quantifications. Coco levels detection range was 125–8000 pg/ml. The inter-assay coefficient of variation (CV) = 7.6%, and the intra-assay CV = 3.9%.

2.4. P19 cell culture and MAP-2 detection

P19 cells (Sigma Aldrich) growing and differentiation protocol are as described elsewhere (Bani-Yaghoub et al., 2000; Penn et al., 2017). BMP-2 and BMP-4 (R&D Systems) were neutralized with 500 ng/ml anti-BMP-2/4, rhCOCO at 500 pg/ml, 1200 pg/ml, 1800 pg/ml, and 5000 pg/ml (R&D Systems). Following 4 days of stimulations the cells were plated in tissue culture-grade dishes and were re-cultured at day 7 in 24-well plates with coverslips (De-Groot Laboratories) at a final concentration of 5×10^4 cells/well. On day 8, the cells were washed with PBS, fixed with 4% paraformaldehyde (Electrone Microscopy Sciences) and permeabilized with 0.5% Triton Tx (Sigma Aldrich). Detection of MAP-2 was performed using staining with anti-rabbit MAP-2 mAb (1:100, D5G1, Cell Signaling), followed by incubation with Alexa Fluor® 488 conjugated IgG (1:1000; Molecular Probes USA) and incubation with Hoechst antibody (1:100; Sigma Aldrich). The coverslips were mounted with Immu-Mount (Thermo Fisher Scientific). Images were obtained by Zeiss 510 confocal microscope. Analysis of % MAP-2^+ cells from total cells was performed by *Image J* software.

2.5. Statistics

The Kruskal-Wallis Test tested the differences between the groups, as Coco values are not normally distributed. Multiple groups comparisons for paramentic results was done by one way ANOVA test and Tukey's post hoc test (alpha = 0.05) for specific comparisons between groups. Student's *t*-test was used to compare paramentic results of 2 groups. Data are presented as mean ± standard error of the means for the Coco levels and by mean and range for % MAP2+ P19 cells.

3. Results

The Coco serum levels were significantly reduced in RR-MS patients (1237.4 ± 157.9 pg/ml) and PP-MS patients (1227.1 ± 480.8 pg/ml) compared to HC (1883.3 ± 243.2 pg/ml) and patients with RA (1833.7 ± 498.7 pg/ml, p = .029, Fig. 1A). The Coco levels of untreated RR-MS (1219.7 ± 195.1 pg/ml) were similar to the levels of

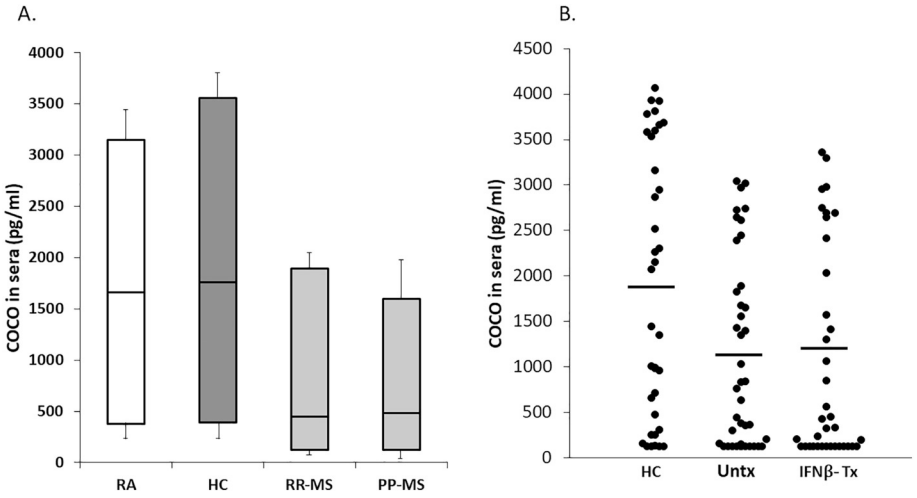

Fig. 1. Reduced levels of Coco in the serum of RR-MS and PP-MS patients.
Coco levels were examined by sandwich ELISA in the serum of HC, RR-MS patients, PP-MS patients and RA patients (A) Coco levels were significantly reduced in the serum of RR-MS and PP-MS patients compared to HC and RA patients. Data are presented as median and inter-quartile ranges. (B) Dot plot presentation of the study group. Lower sera levels of Coco was found in untreated patients with RR-MS and IFN-β-treated RR-MS patients as compared to HC.

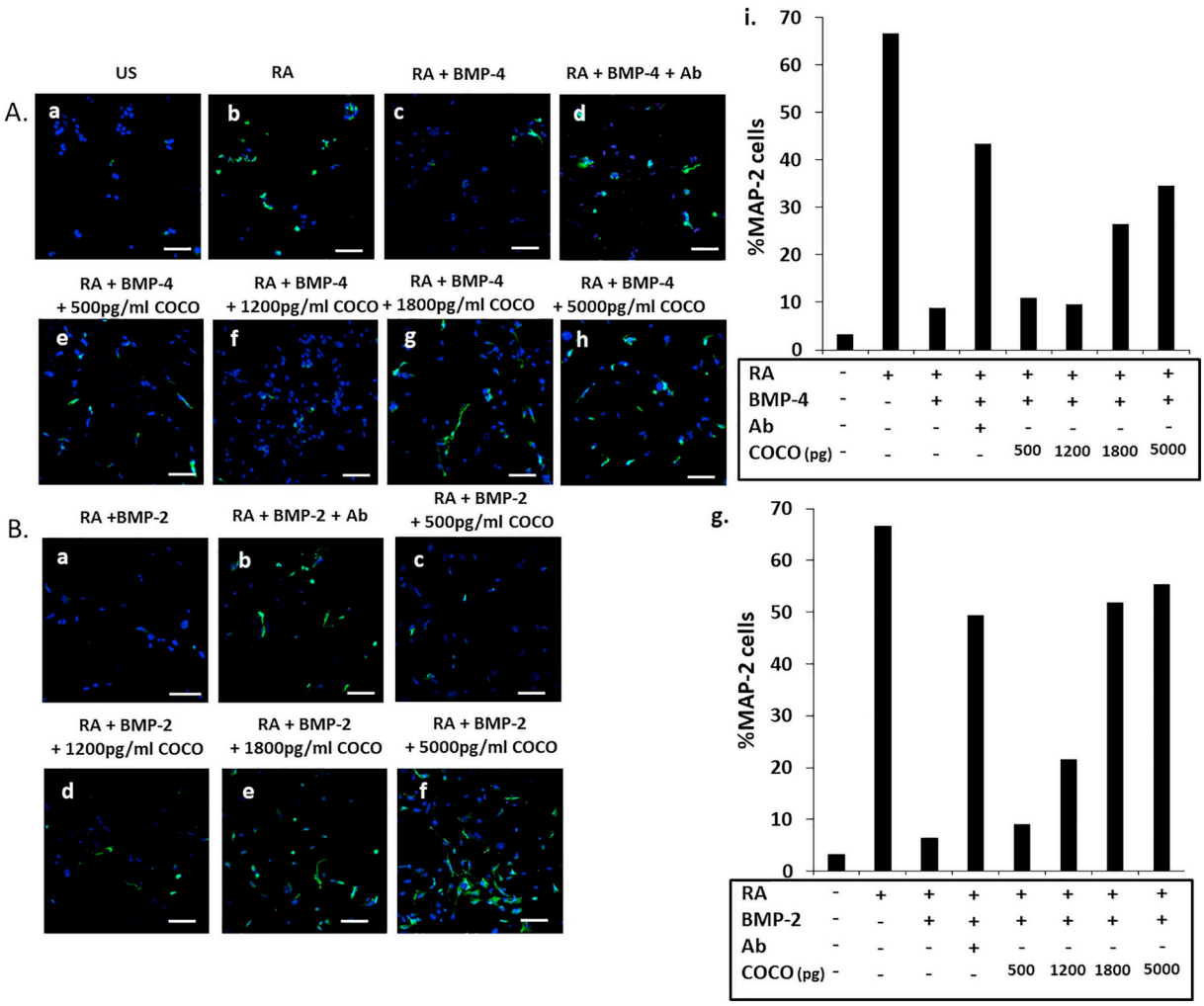

Fig. 2. The ability of Coco to inhibit BMP-4 and BMP-2 effect on neurogenesis.
P19 cells were incubated for 4 days with one of the following conditions/stimulations in part A: (a) none (b) 5×10^{-7}M all-*trans*-retinoic acid, (c) retinoic acid + 5 ng/ml rhBMP-4, (d) retinoic acid + BMP-4 + anti-BMP-2/4 mAb, (e) retinoic acid + BMP-4 + 500 pg/ml (f) retinoic acid + BMP-4 + 1200 pg/ml rhCoco (which is the average sera level of Coco in RR-MS patients), (g) retinoic acid + BMP-4 + 1800 pg/ml rhCoco (which is the average sera level of Coco in HC), (h) retinoic acid + BMP-4 + 5000 pg/ml (h) rhCoco. (i) Bar graph that summarized the average of %MAP-2-positive cells from total cells. Part B: (a) retinoic acid + 5 ng/ml rhBMP-2, (b) retinoic acid + 5 ng/ml rhBMP-2 + anti-BMP-2/4 mAb (c) retinoic acid + 5 ng/ml rhBMP-2 + 500 pg/ml rhCoco, (d) retinoic acid A + 5 ng/ml rhBMP-2 + 1200 pg/ml, (e) retinoic acid A + 5 ng/ml rhBMP-2 + 1800 pg/ml, (f) retinoic acid + 5 ng/ml rhBMP-2 + 5000 pg/ml. (g) Bar graph that summarized the average of %MAP-2-positive cells from total cells. Duplicates were made and 3–5 images were collected from each sample. On day 4, all cultures were re-plated without stimulations. MAP-2^+ cells were examined on day 8. Quantification of %MAP-2^+ cells for each treatment demonstrates a significant increase in %MAP-2 cells in response to 1800 pg/ml rhCoco, compared to 1200 pg/ml rhCoco, in the presence of either retinoic acid + rhBMP-4 or rhBMP-2, scale bar = 100 μm.

IFN-β-treated RR-MS patients (1256.9 ± 256.2 pg/ml) and were significantly lower as compared to HC ($p = .041$, Fig. 1B). Coco was under the sensitivity threshold of detection (125 pg/ml) in the CSF of RR-MS patients and controls, as well as in the 24 or 48 h supernatants of unstimulated and stimulated PBMCs with anti-CD3/CD28 or with LPS. As there were differences in the gender distribution of patients with PP-MS and those with RA, we compared the sera Coco levels of females vs. males in all groups and did not find significant differences.

We used P19 cells, which differentiate into neurons in the presence of retinoic acid, to examine the biological relevance of the differences in sera Coco levels. Coco is known to inhibit BMP-4 (Bell et al., 2003). Due to the high amino acid sequence homology (86%) of human BMP-2 and BMP-4 (Celeste et al., 1990), we examined also the potential of Coco to hinder BMP-2 effect. We examined the phenotype of P19 cells in the presence of retinoic acid and BMP-4 or BMP-2 in different doses of rhCoco according to the average sera-Coco concentration of RR-MS patients (~1200 pg/ml) and of HC (~1800 pg/ml) (Fig. 2Ai, Bg).

We compared the different conditions of P19 cells as follow: no stimulation, with retinoic acid, retinoic acid + BMP-4, with retinoic acid + anti BMP-2/4 mAb, with retinoic acid + BMP-4 + 500 pg/ml rhCoco, with retinoic acid + BMP-4 + 1200 pg/ml rhCoco, with retinoic acid + BMP-4 + 1800 pg/ml rhCoco, and with retinoic acid + BMP-4 + 5000 pg/ml rhCoco. There was a statistically significant difference between these groups as determined by one-way ANOVA ($F(7,24) = 44.33$, $p < .001$). Tukey's post hoc test (alpha = 0.05) revealed that the negligible %MAP-2^+ cells that were detected without stimulation (3.2, range: 0–4.8%MAP-2^+ cells, Fig. 2Aa) was significantly lowers vs. addition of retinoic acid to the P19 cells. MAP-2^+ cells (66.6, range: 60.5–72.5%MAP-2^+ cells, Fig. 2Ab, $p < .001$). The addition of rhBMP-4 significantly reduced the %MAP-2^+ cells (8.8%, range: 6.0–10.8%MAP-2^+ cells, Fig. 2Ac, $p < .001$), and addition of anti- BMP-2/4 mAb abolished the BMP-4 effect (43.4%, range 35.0–52.9%MAP-2^+ cells, Fig. 2Ad, $P < .001$). Addition of 500 pg/ml rhCoco in the presence of retinoic acid and BMP-4 (10.9%, range: 5.8–13.9%MAP-2^+ cells, Fig. 2Ae) did not affect the % MAP-2^+ cells vs. retinoic acid + BMP-4 ($p = N{\cdot}S$). Similar %MAP-2^+ cells was

observed with 1200 pg/ml rhCoco (12.1%, range: 6.1–12.1%MAP-2$^+$ cells, Fig. 2Af, p = N·S vs. 500 pg/ml rhCoco). A significant increase in %MAP-2$^+$ cells was observed with 1800 pg/ml rhCoco (26.6% range: 21.4–31.0%MAP-2$^+$ cells, Fig. 2Ag, vs. retinoic acid + BMP-4, p = .003 and vs. retinoic acid + BMP-4 + 1200 pg/ml rhCoco, p = .007). Addition of 5000 pg/ml rhCoco further induced the %MAP-2$^+$ cells but without significant difference vs. 1800 pg/ml rhCoco (34.6%, range 25.9–46.2%MAP-2$^+$ cells, Fig. 2Ah).

In similar experiment with rhBMP-2 (Fig. 2Ba-g), There was a statistically significant difference between the following groups as determined by one-way ANOVA ($F(7,14) = 51.16$, $p < .001$). Tukey's post hoc test (alpha = 0.05) revealed that there was a significant increase in %MAP-2$^+$ cells expression with 1800 pg/ml rhCoco (51.9, range: 46.7–60.7%MAP-2 cells, Fig. 2Be) vs. 1200 pg/ml rhCoco (21.5, range: 13.6–28.6%MAP-2 cells, Fig. 2Bd, P < .001). Exposure to 5000 pg/ml rhCoco was associated 55.3%MAP-2$^+$ cells (range 53.3–57.4%MAP-2 cells, Fig. 2Bf, P < .001 vs. 1200 pg/ml rhCoco, P = N·S vs. 1800 pg/ml of Coco). The %MAP-2$^+$ cells expression with 500 pg/ml rhCoco was 9.2, range: 6.8–12.7%MAP-2$^+$ cells (Fig. 2Bc, p < .001 vs. 1800 pg/ml rhCoco and vs. 5000 pg/ml rhCoco).

Interestingly, %MAP-2 expression with retinoic acid +1800 pg/ml rhCoco but with BMP-2 was significantly higher than with BMP-4 (p = .003, Student's *t*-test). The fold induction of MAP-2$^+$cells with 1800 pg/ml rhCoco and BMP-2 was 5.9, compared to 3.1 in the presence of BMP-4, suggesting an increased potential of Coco to inhibit BMP-2 compared to BMP-4.

4. Discussion

Unlike the BMP antagonists noggin and follistatin, but similar to DAN, another DAN family member, Coco was not detected in PBMC supernatants. Coco was not detected in CSF of both RR-MS patients and controls. This is in contrast to DAN, which is markedly present in CSF of RR-MS patients (Mausner-Fainberg et al., 2016), but like BMP2, BMP4 and BMP5 that were detected in the sera but not in the CSF of patients with RRMS and controls (Penn et al., 2017). Since BMPs are expressed in the CNS of patients with multiple sclerosis (Deininger et al., 1995), the lack of detection of BMP and coco in CSF does not necessarily indicate their inactivity in CNS of patients with multiple sclerosis.

Similarly, to DAN levels, Coco sera levels of RR-MS patients were lower than in HC. Reduced levels of the DAN family members further support the notion of dysregulated BMP signaling in MS.

In contrast to DAN, which is induced by IFN-β, sera Coco levels were not altered with this treatment. This observation supports the existence of different regulation mechanisms for different BMP antagonists. The average Coco level in sera of HC but not of MS patients hindered the effect of the BMP-4 and BMP-2 by differentiating P19 cell into MAP-2$^+$ cells. Our results indicate that the decreased Coco sera levels may have a biological meaning on the BMPs regulation in MS. This biological effect was studied in a neurogenic model rather than a specific oligodendrogenesis/remyelination model.

There are only few reports about BMP-2 antagonism by Coco (Gao et al., 2012). Our results indicate that BMP-2 may be an additional ligand for Coco antagonism.

In conclusion, we demonstrated that reduced sera Coco levels in MS patients may have a role in BMP-2 and BMP-4 dysregulation, and therefore raises the possibility that the levels of Coco are related to the oligodendrogenesis inhibition in MS lesions. However, without direct evidences, it is difficult to draw a definite conclusion regarding the role and significance of reduced serum level of Coco to oligodendrogenesis.

Declarations of interest

None for all authors.

Funding

Merck KGaA supported the study (grant no. MS200136_0049).

Acknowledgements

We thank Esther Eshkol, institutional medical copyeditor, for editorial assistance.

References

Ara, J., See, J., Mamontov, P., Hahn, A., Bannerman, P., Pleasure, D., Grinspan, J.B., 2007. Bone morphogenetic proteins 4, 6, and 7 are up-regulated in mouse spinal cord during experimental autoimmune encephalomyelitis. J. Neurosci. Res. 86, 125–135. https://doi.org/10.1002/jnr.21462.

Bani-Yaghoub, M., Felker, J.M., Sans, C., Naus, C.C.G., 2000. The effects of bone morphogenetic protein 2 and 4 (BMP2 and BMP4) on gap junctions during neurodevelopment. Exp. Neurol. 162, 13–26. https://doi.org/10.1006/exnr.2000.7294.

Bell, E., Muñoz-Sanjuán, I., Altmann, C.R., Vonica, A., Brivanlou, A.H., 2003. Cell fate specification and competence by Coco, a maternal BMP, TGFbeta and Wnt inhibitor. Development 130, 1381–1389. https://doi.org/10.1242/dev.00344.

Celeste, A.J., Iannazzi, J.A., Taylor, R.C., Hewick, R.M., Rosen, V., Wang, E.A., Wozney, J.M., 1990. Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. Proc. Natl. Acad. Sci. U. S. A. 87, 9843–9847. https://doi.org/10.1073/pnas.87.24.9843.

Chang, A., Tourtellotte, W.W., Rudick, R., Trapp, B.D., 2002. Premyelinating Oligodendrocytes in Chronic Lesions of Multiple Sclerosis. N. Engl. J. Med. 346, 165–173. https://doi.org/10.1056/NEJMoa010994.

Deininger, M., Meyermann, R., Schluesener, H., 1995. Detection of two transforming growth factor-beta-related morphogens, bone morphogenetic proteins-4 and -5, in RNA of multiple sclerosis and Creutzfeldt-Jakob disease lesions. Acta Neuropathol. 90, 76–79.

Fagerberg, L., Hallström, B.M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., Habuka, M., Tahmasebpoor, S., Danielsson, A., Edlund, K., Asplund, A., Sjöstedt, E., Lundberg, E., Szigyarto, C.A., Skogs, M., Takanen, J.O., Berling, H., Tegel, H., Mulder, J., Nilsson, P., Schwenk, J.M., Lindskog, C., Danielsson, F., Mardinoglu, A., Sivertsson, A., von Feilitzen, K., Forsberg, M., Zwahlen, M., Olsson, I., Navani, S., Huss, M., Nielsen, J., Ponten, F., Uhlén, M., 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol. Cell. Proteomics 13, 397–406. https://doi.org/10.1074/mcp.M113.035600.

Fuller, M.L., DeChant, A.K., Rothstein, B., Caprariello, A., Wang, R., Hall, A.K., Miller, R.H., 2007. Bone morphogenetic proteins promote gliosis in demyelinating spinal cord lesions. Ann. Neurol. 62, 288–300. https://doi.org/10.1002/ana.21179.

Gao, H., Chakraborty, G., Lee-Lim, A.P., Mo, Q., Decker, M., Vonica, A., Shen, R., Brogi, E., Brivanlou, A.H., Giancotti, F.G., 2012. The BMP inhibitor Coco reactivates breast cancer cells at lung metastatic sites. Cell. https://doi.org/10.1016/j.cell.2012.06.035.

Gross, R.E., Mehler, M.F., Mabie, P.C., Zang, Z., Santschi, L., Kessler, J.A., 1996. Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron 17, 595–606. https://doi.org/10.1016/S0896-6273(00)80193-2.

Hsu, D.R., Economides, A.N., Wang, X., Eimon, P.M., Harland, R.M., 1998. Identifies a novel family of secreted proteins that antagonize BMP activities. Cell 1, 673–683.

Kuhlmann, T., Miron, V., Cuo, Q., Wegner, C., Antel, J., Brück, W., 2008. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 131, 1749–1758.

Lassmann, H., Brück, W., Lucchinetti, C., Rodriguez, M., 1997. Remyelination in multiple sclerosis. Mult. Scler. J. 3, 133–136. https://doi.org/10.1177/135245859700300213.

Mabie, P.C., Mehler, M.F., Marmur, R., Papavasiliou, A., Song, Q., Kessler, J. a, 1997. Bone morphogenetic proteins induce astroglial differentiation of oligodendroglial-astroglial progenitor cells. J. Neurosci. 17, 4112–4120. https://doi.org/10.1523/JNEUROSCI.17-11-04112.1997.

Mausner-Fainberg, K., Urshansky, N., Regev, K., Auriel, E., Karni, A., 2013. Elevated and dysregulated bone morphogenic proteins in immune cells of patients with relapsing-remitting multiple sclerosis. J. Neuroimmunol. 264, 91–99. https://doi.org/10.1016/j.jneuroim.2013.09.004.

Mausner-Fainberg, K., Kolb, H., Penn, M., Regev, K., Vaknin-Dembinsky, A., Gadoth, A., Kestenbaum, M., Karni, A., 2016. Differential screening-selected gene aberrative in neuroblastoma (DAN) is increased in the CSF of patients with MS and may be induced by therapy with interferon-β. J. Neuroimmunol. 292, 93–96. https://doi.org/10.1016/j.jneuroim.2016.01.019.

Mira, H., Andreu, Z., Suh, H., Lie, D.C., Jessberger, S., Consiglio, A., San Emeterio, J., Hortigüela, R., Marqués-Torrejón, M.A., Nakashima, K., Colak, D., Götz, M., Fariñas, I., Gage, F.H., 2010. Signaling through BMPR-IA regulates quiescence and long-term activity of neural stem cells in the adult hippocampus. Cell Stem Cell 7, 78–89. https://doi.org/10.1016/j.stem.2010.04.016.

Pearce, J.J.H., Penny, G., Rossant, J., 1999. A mouse cerberus/Dan-related gene family. Dev. Biol. 209, 98–110. https://doi.org/10.1006/dbio.1999.9240.

Penn, M., Mausner-Fainberg, K., Golan, M., Karni, A., 2017. High serum levels of BMP-2 correlate with BMP-4 and BMP-5 levels and induce reduced neuronal phenotype in

patients with relapsing-remitting multiple sclerosis. J. Neuroimmunol. 310, 120–128. https://doi.org/10.1016/j.jneuroim.2017.07.008.

Urshansky, N., Mausner-Fainberg, K., Auriel, E., Regev, K., Bornstein, N.M., Karni, A., 2011a. Reduced production of noggin by immune cells of patients with relapsing-remitting multiple sclerosis. J. Neuroimmunol. 232, 171–178. https://doi.org/10.1016/j.jneuroim.2010.10.007.

Urshansky, N., Mausner-Fainberg, K., Auriel, E., Regev, K., Karni, A., 2011b. Low and dysregulated production of follistatin in immune cells of relapsing-remitting multiple sclerosis patients. J. Neuroimmunol. 238, 96–103. https://doi.org/10.1016/j.jneuroim.2011.08.003.

Wolswijk, G., 1998. Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. J. Neurosci. 18, 601 LP–609.

Zhou, S., Flamier, A., Abdouh, M., Tetreault, N., Barabino, A., Wadhwa, S., Bernier, G., 2015. Differentiation of human embryonic stem cells into cone photoreceptors through simultaneous inhibition of BMP, TGF and Wnt signaling. Development. https://doi.org/10.1242/dev.125385.