Adult hippocampus derived soluble factors induce a neuronal-like phenotype in mesenchymal stem cells☆

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Abstract

Bone marrow-derived mesenchymal stem cells (MSCs) are not restricted in their differentiation fate to cells of the mesenchymal lineage. They acquire a neural phenotype *in vitro* and *in vivo* after transplantation in the central nervous system. Here we investigated whether soluble factors derived from different brain regions are sufficient to induce a neuronal phenotype in MSCs. We incubated bone marrow-derived MSCs in conditioned medium (CM) derived from adult hippocampus (HCM), cortex (CoCM) or cerebellum (CeCM) and analyzed the cellular morphology and the expression of neuronal and glial markers. In contrast to muscle derived conditioned medium, which served as control, conditioned medium derived from the different brain regions induced a neuronal morphology and the expression of the neuronal markers GAP-43 and neurofilaments in MSCs. Hippocampus derived conditioned medium had the strongest activity. It was independent of NGF or BDNF; and it was restricted to the neuronal differentiation fate, since no induction of the astroglial marker GFAP was observed. The work indicates that soluble factors present in the brain are sufficient to induce a neuronal phenotype in MSCs.

Keywords: Differentiation; Stem cells; Hippocampus; Neuron

Mesenchymal stem cells (MSCs) derived from bone marrow stroma give rise to a variety of mesenchymal cell types, such as osteocytes, chondrocytes and adipocytes [13]. MSCs can also differentiate into cells from the neuro-ectodermal lineage such as neurons, astrocytes and oligodendrocytes (for review see [18]). Recently, some studies focused on the search of physiological inductors for neural differentiation of MSCs. These required the presence of growth factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) or epidermal growth factor, or the direct contact of MSCs with neural tissue, either in transplantation or co-culture experiments [1,3,7,9–11,17]. For example, in a co-culture system with postnatal hippocampal tissue, MSCs displayed neuronal morphology and expressed the neuronal marker NeuN [1]. These data suggest that the MSC-neural microenvironment affects the fate of MSCs inducing a neural-like phenotype. The MSC-neural microenvironment consists of cell–cell and cell–matrix interactions, and of soluble factors. The essential components are not known, but direct contact between MSCs and neural cells may be required for the induction of a neuronal phenotype of MSCs [1].

Here, we investigated the interactions between MSCs and adult neural microenvironment, specifically of the hippocampus, cortex and cerebellum. By using conditioned medium derived from the different adult brain regions, we focused on effects of soluble factors on MSC fate, proliferation and survival.

All experiments were performed according to the European Communities Council Directive (86/609/EEC) and the local institutional guidelines on animal care and ethics (INTA, Universidad de Chile). MSCs were isolated from 5 weeks old Wistar rat femurs and tibias, and adherent cells were grown according to standard methods in minimum essential medium (MEM) containing 10% Fetal Bovine Serum (FBS) (Gibco Invit-

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rogen, Pasley, UK) at a seeding cell density of 8000 cells/cm^2 [19]. After 3–5 days, the resulting monolayer of cells, hereafter named bone marrow-derived mesenchymal stem cells, was trypsinized and aliquots were frozen and stored or further cultivated. Growth factors and blocking reagents used were 100 ng/ml NGF (R&D Systems, Germany), 10 ng/ml BDNF (R&D Systems, Germany), 100 μg/ml anti-NGF and 10 μg/ml anti-BDNF (gifts from Georg Dechant, University of Innsbruck). All reagents were confirmed to be active by a dorsal root ganglion bioassay [2].

Osteogenic differentiation on MSCs from passage 2–4 was induced by MEM-10% FBS supplemented with 0.1 μM dexamethasone, 10 mM β-glycerophosphate (Sigma), and 50 μM L-ascorbic acid-2-phosphate (Sigma), revealed after 4 weeks by hydroxyapatite mineralization and detected by microscopy after staining with 40 mM Alizarin Red S (Sigma, Saint Louis Missouri) [14]. MSC adipogenic differentiation was induced after incubation in MEM-10% FBS supplemented with 1 μM dexamethasone (Sigma), 10 μg/ml insulin, 0.5 mM 3-isobutyl-1-methylxanthine (Sigma) and 100 μM indomethacin (Sigma). After 1 week, the adipogenic differentiation was confirmed by staining of cytoplasmatic lipid droplets with saturated Oil Red O (Sigma) [6].

Adult Wistar rats (200–400 g) were anesthetized, sacrificed and the hippocampus, cerebellum and cortex removed under sterile conditions. In addition, the Tibialis Anterior muscles were also dissected. Tissues were weighed and placed into a 15 ml tube to which 2 ml fresh MEM was added. After incubating with gentle stirring (to avoid tissue damage) (2 h, 4 °C), the soluble fraction was collected (18,000 × *g*, 30 min, 4 °C) and filtered through a 0.2 μm filter. The different tissue soluble factions were analyzed for protein content by the Bradford assay and stored at −80 °C. Cells were incubated with tissue-conditioned media (200 μg/ml of protein) and MEM-0.5% FBS.

For proliferation studies, MSCs suspended in MEM-10% FBS were plated in 24- or 4 poly-L-lysine coated multiwell plates (2500 cells/cm^2) [4]. Cells were incubated for 24 h and switched to the control (MEM-0.5% FBS) or hippocampus conditioned media. At various time intervals, adherent cells were washed (2×) with phosphate buffered saline (PBS), trypsinized and the number of viable cells was determined by Trypan blue exclusion.

Cells grown on poly-L-lysine coated glass coverslips were fixed with 4% paraformaldehyde in PBS. Fixed cells were treated with methanol for 10 min at −20 °C and 0.1% Triton X-100 for 10 min at room temperature for permeabilization. After two washing steps, cells were treated with PBS-5% FBS (blocking solution) for 1 h at room temperature. Later, cells were incubated for 2 h at room temperature in antibody-containing blocking solution. The following primary antibodies were used: NF-H (1/500) (Sigma); GFAP (1/400) (Sigma); GAP-43 (1/20) (Sta. Cruz Biotechnology, California); NeuroD (1/100) (Sta. Cruz Biotechnology); β-III tubulin (1:250; Promega, USA). After 4 washing steps, cells were incubated with secondary antibodies (anti-Goat FITC and anti-Mouse FITC (1/250 and 1/150, respectively) (Sta. Cruz Biotechnology) diluted in blocking solution for 2 h at room temperature. Nuclear counterstaining was done with Hoechst 33240 (1 μg/ml bisbenzimide in PBS-3% FBS, Sigma). After the final wash, samples were mounted on slides using Fluoromount-G (Southern Biotechnology Associates Inc., USA). For quantification, the numbers of immunoreactive cells were determined by counting specifically stained cells in at

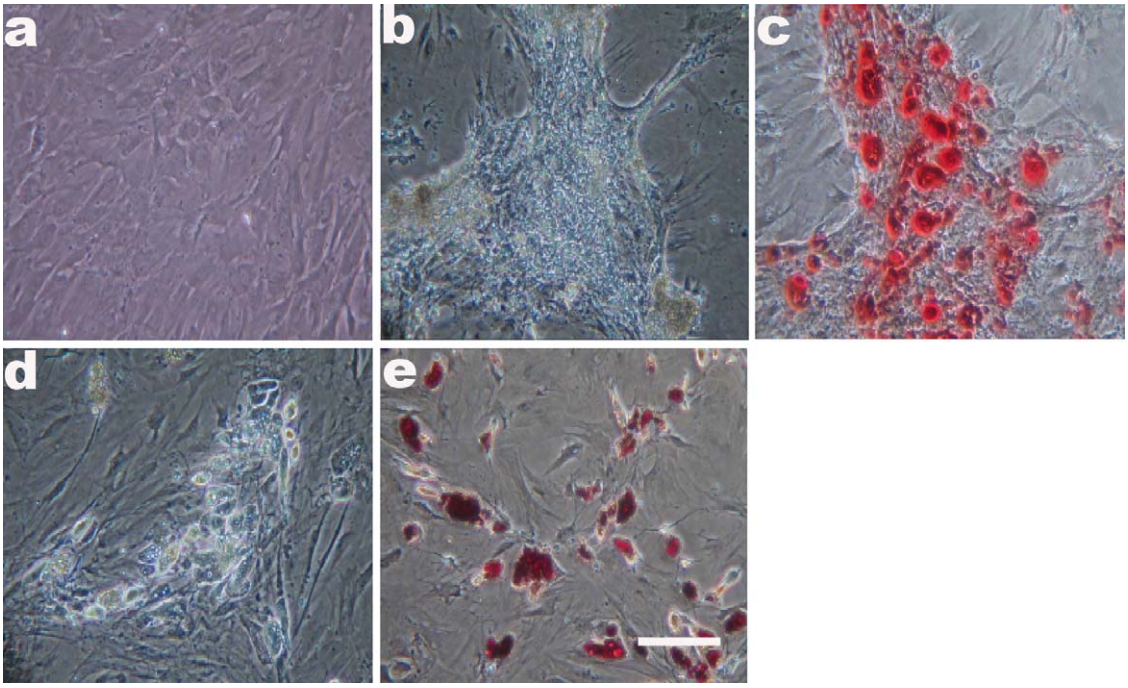

Fig. 1. MSC differentiation potential. MSCs were incubated in control medium (MEM-10% FBS) (a), osteogenic medium (b, c) or adipogenic medium (d, e) and analyzed for their differentiation potential. (a, b, d) Phase contrast images. (c) MSCs in osteogenic medium and staining with Alizarin red. (e) MSCs in adipogenic medium and staining with Oil Red O. Scale bar = 125 μm. MSCs used are at least bipotent cells, since they are able to differentiate to osteoblasts and adipocytes.

least 10 random fields (200–300 cells/experiment) on the coverslip using a fluorescent microscope (Olympus or Leika DMR). Experiments were done in triplicate. Error bars represent the S.D.

RT-PCR was performed on mRNA isolated from MSCs (RNeasy® Kit, Qiagen, Germany). RT-PCR primers were GAPDH 5′-GGT CGG TGT GAA CGG ATT TG-3′ and 5′-GTG AGC CCC AGC CTT CTC CAT-3′, TrkA 5′-AGA GAG CAT CCT CTA CCG C-3′ and 5′-ACT CGA TCG CCT CAG TGT TG-3′, and TrkB 5′-CGA ATC TCC AAC CTC AGA CCA CC-3′ and 5′-AAC TTG AGC AGA AGC AGC ATC ACC-3′. PCR conditions were 94 °C, 2 min, follow by 94 °C, 45 s and 60 °C, 1 min; then 72 °C, 2 min, for a total of 40 cycles.

Means are expressed with their standard deviations. For NF-H expression 1-way ANOVA was used followed by Tukey's post hoc test. For proliferation and survival curves 2-way ANOVA was used. Statistical analysis was performed using SYSTAT 11.

To confirm the identity and the differentiation potential of MSCs used in the present study, cells isolated from bone marrow stroma were (i) analyzed by FACS for their homogeneity and (ii) incubated in specific differentiation media. MSCs contained only a minor fraction (0.9%) of CD45 positive hematopoietic lineage cells (data not shown), and can therefore be considered as virtually pure. Under proliferation conditions MSCs were adherent with a fibroblast-like morphology (Fig. 1a). After 3 weeks of incubation in osteogenic medium, MSCs formed a confluent cell layer and synthesized hydroxyapatite, denoting extracellular matrix mineralization and development of osteoblastic cells (Fig. 1b and c). For adipogenic differentiation, MSCs were exposed to adipogenic medium for 1 week. Cells accumulated intracellular neutral lipid droplets indicating an adipocyte phenotype (Fig. 1d and e). These results demonstrate that the cells used in the present study have MSC properties.

We assessed if adult brain tissue contains soluble factors that induce a neuronal-like phenotype in MSCs. Therefore, we incubated MSCs for 3 days in conditioned medium (CM) derived from adult hippocampus (HCM), cortex (CoCM) or cerebellum (CeCM). CM derived from muscle (MCM) and medium alone served as controls. The effects were assessed by analyzing the appearance of cells with a neuronal-like morphology and the expression of neural lineage markers.

Only CMs from brain tissues were able to promote a morphological change in MSCs. We observed a switch from a fibroblast-like morphology into cells with bipolar and multipolar processes (Fig. 2a). In addition, CMs from the different brain regions, but not from muscle tissue, induced expression of the neuronal gene NF-H in MSCs (Fig. 2b). The strongest effect in induction of neuronal-like morphology and in expression of NF-H was displayed by HCM (Figs. 2 and 3, $p < 0.005$). While more than 40% of MSCs expressed NF-H after stimulation with HCM, only 11.4% and 17.6% of MSCs expressed NF-H after stimulation with CeCM or CoCM, respectively (Fig. 3a). Therefore, and since the hippocampus has ongoing neurogenesis in the adult, the effects of HCM were analyzed in more detail by immunostaining for the neuronal markers NeuroD, β-III tubulin, NeuN, NF-H, GAP-43, the glial marker GFAP, and by quantitative analysis of the cells expressing these markers. Under either control or HCM conditions, more than 70% of MSCs expressed the neuronal determination marker NeuroD and the young neuronal marker β-III tubulin. Very few cells (<1%) expressed NeuN under these conditions. When stimulated for 3 days with HCM, we observed a dramatic difference in the percentage of cells expressing NF-H (<1% in control, 41.1% in HCM; $p < 0.001$) (Fig. 3a) and approximately 5% express GAP-43. In contrast, the astrocytic marker GFAP was not expressed by MSCs irrespectively of the conditions used, indicating that HCM does not

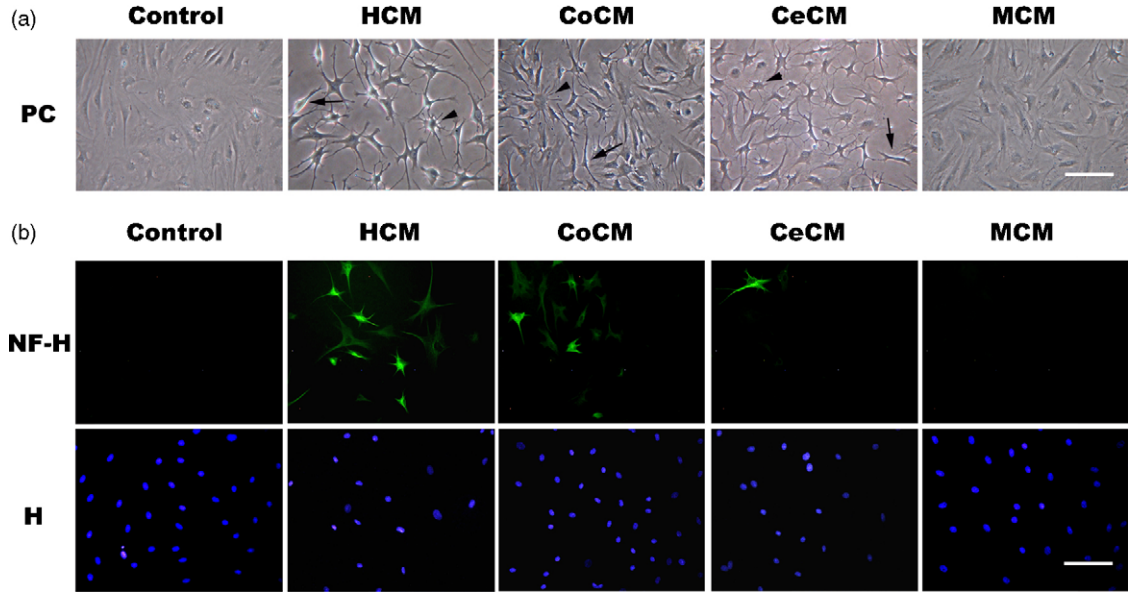

Fig. 2. Soluble factors from different brain regions induce the expression of NF-H in MSCs. MSCs were incubated for 3 days in control medium (MEM-0.5% FBS), HCM, CoCM, CeCM or MCM and analyzed for NF-H expression by immunofluorescence. (a) Phase contrast images showing the morphological differences. Cells with a bipolar (arrows) and with multipolar (arrowheads) morphology are present only in HCM, CoCM and CeCM. (b) Fluorescence images for NF-H (green) and Hoechst (blue) are shown. NF-H-positive cells are present only in HCM, CoCM and CeCM. Scale bars = 125 μm.

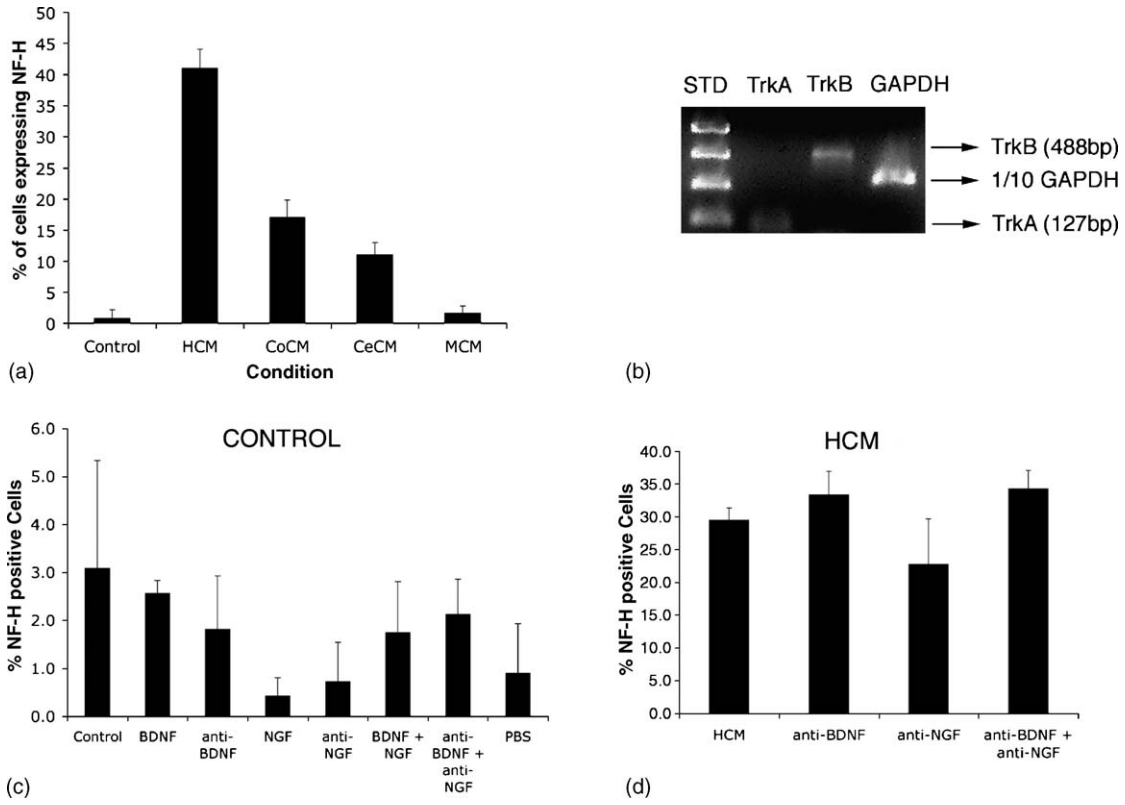

Fig. 3. Quantitative analysis of NF-H expression in MSCs. MSCs were incubated for 3 days in control medium (MEM-0.5% FBS), HCM, CoCM, CeCM, MCM, NGF, BDNF, anti-NGF or anti-BDNF and analyzed for NF-H expression by immunofluorescence. The percentages of NF-H-positive cells were determined. (a) Quantitative analysis of the effects of the different CMs; (b) RT-PCR demonstrating mRNA expression of TrkA and TrkB by MSCs; (c) no effects of NGF and BDNF on NF-H expression; and (d) no effects of anti-NGF and anti-BDNF on the HCM activity. Experiments were done in triplicate. Error bars represent S.D. and 1-way ANOVA followed by Tukey's post hoc test was used for statistical analysis.

induce an astrocyte-like phenotype in MSCs. Taken together, these results suggest that hippocampal soluble factors induce a neuronal-like phenotype on MSCs.

In further experiments, we tested if the neurotrophins NGF and BDNF as candidate molecules were involved in the effect of HCM on NF-H expression in MSCs. First, we confirmed that MSCs do express the mRNAs for the NGF and BDNF receptors TrkA and TrkB, respectively (Fig. 3b). Next, we tested whether NGF and BDNF have a similar effect on NF-H expression in MSCs as HCM. Therefore, we stimulated MSCs for 3 days with NGF and/or BDNF, and analyzed the cells for NF-H expression by immunocytochemistry. Neither NGF nor BDNF had any detectable effect on NF-H expression (Fig. 3c). Finally, we tested for the presence of NGF or BDNF activity in the HCM by using blocking antibodies. MSCs were incubated for 3 days with HCM in the presence or absence of blocking antibodies and subsequently, expression of NF-H was analyzed. The presence of blocking antibodies against NGF and BDNF did not inhibit the HCM activity on NF-H expression in MSCs (Fig. 3d). This strongly suggests that NGF and/or BDNF is/are not sufficient to explain the hippocampus derived activity on NF-H expression in MSCs.

In addition to the effects on morphology and expression of neuronal markers, HCM promoted a significant increase on the expansion, over time, of MSC cultures compared to the control condition (Fig. 4a, $p < 0.001$). Specifically, the number of cells

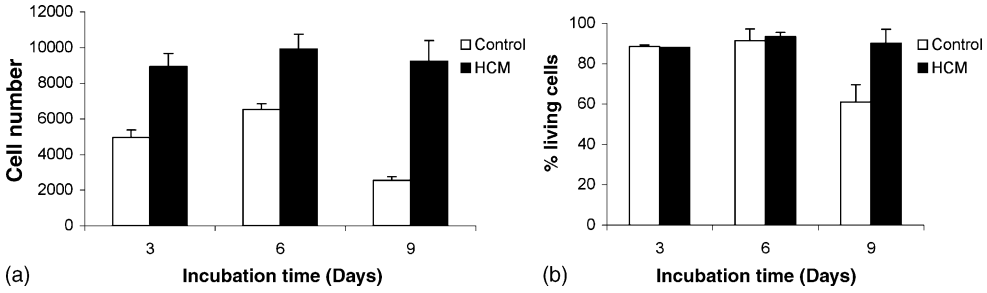

Fig. 4. Hippocampal soluble factors promote expansion and survival of MSCs. MSCs were incubated for 9 days in control medium (MEM-0.5% FBS) or in HCM. Total number (a) and percentage (b) of living cells were determined at day 3, day 6 and day 9. Note the early and significant increase and the protracted maintenance in cell number in HCM. Experiment was done in triplicate. Error bars represent S.D. and 2-way ANOVA was used for statistical analysis.

after 3 days in HCM was about 80% higher compared to the control (Fig. 4a). After that, the cell number in HCM was maintained, whereas under control conditions, a significant decrease was observed between days 6 and 9 (Fig. 4a). In addition to this, HCM prevented MSCs from substrate detachment and cell death, over time that occurred when cells were grown under control conditions (Fig. 4b, $p < 0.05$). Accordingly, at day 9, viability was $91 \pm 7\%$ and $61 \pm 8\%$ for cells incubated in the presence and absence of HCM, respectively. Thus, the hippocampus derived CM promoted MSC expansion and survival.

The present work demonstrates that soluble factors derived from different brain tissues induce a neuronal-like morphology and the expression of neuronal markers in adult MSCs. We further showed that this diffusible factor activity was particularly concentrated in a neurogenic structure, i.e. the hippocampus. We could exclude that NGF and/or BDNF were the factors involved in MSC early induction phase of neuronal-like morphology and expression. Therefore, the exact nature of the neuronal-inducing factor(s) released by the nervous tissue, especially by neurogenic structures, remains a very intriguing question. The identification and characterization of these soluble factors will have consequences not only for our understanding of MSC biology, but also for the field of neurogenesis and stem cell fate regulation in general.

Throughout the work we use the expression "neuronal-like", since the data are based solely on morphological and marker expression criteria, and not on data showing functional electrical activity. Several groups have demonstrated that, after transplantation into brain tissue, MSCs migrate and differentiate into cells expressing neural markers including NeuN, NF, tyrosine hydroxylase, MAP-2 and GFAP [3,5,7,9–11,15,22]. The underlying mechanisms were investigated using co-culture systems with postnatal hippocampal tissue and MSCs, suggesting that direct contact between MSCs and neural cells is required for the induction of a neuronal phenotype of MSCs [1]. In co-cultures without direct cell–cell contact, MSCs did not express the late neuronal marker NeuN [1]. In contrast to the present work, where a panel of both early and late neural markers were used, the study by Abouelfetouh et al. [1] used solely the late neuronal marker NeuN to assess neuronal differentiation. Consistently with that study, we did not find a higher number of NeuN positive MSCs in brain tissue-conditioned medium versus the control. However, the expression of other neuronal markers such as NF-H and GAP-43 was induced in MSCs after stimulation with conditioned medium derived from adult hippocampus, suggesting that hippocampus derived soluble factors are sufficient to induce a neuronal-like phenotype in MSCs. The neuronal-like induction activity observed in this study seems to be restricted to neural tissues, since muscle derived soluble factors did not induce morphological changes or the expression of neural markers in MSCs.

In comparison with cerebellum and cortex, hippocampus derived CM was most effective in promoting the neuronal-like phenotype in MSCs. The adult hippocampus is a brain region with ongoing neurogenesis even in the adult [8] making it a potentially rich source of signals promoting neuronal differentiation. For example, hippocampal astrocytes release soluble factors that induce neuronal differentiation of adult neural progenitor cells [20]. Neurotrophins such as NGF or BDNF might have been good candidate molecules for the hippocampal derived factors(s). However, cultures in presence of NGF and/or BDNF, as well as experiments using blocking antibodies, argue against the involvement of these two neurotrophins in the HCM effect.

Apparently, similar to the co-culture experiments by Abouelfetouh et al. and the work by Wehner et al. [21], the activities present in the brain-derived CM do not induce astroglial differentiation, thereby excluding gliogenic activities. This is in contrast to some MSCs—brain transplantation experiments [3,5,7,9–11,22], where neuronal and astroglial differentiation was observed, suggesting the relevance of exposure to a versatile environment with glio- and neurogenic activities in the brain, as opposed to the *in vitro* situation.

In conclusion, MSCs can be induced *in vitro* to express a neuronal phenotype without non-physiological compounds or direct contact with the neural environment. Since non-physiological agents used to induce neuronal differentiation in MSCs have negative side-effects, like changes in chromatin structure or apoptosis [12,16], the present study may be relevant for future transplantation research.

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References

[1] A. Abouelfetouh, T. Kondoh, K. Ehara, E. Kohmura, Morphological differentiation of bone marrow stromal cells into neuron-like cells after co-culture with hippocampal slice, Brain Res. 1029 (2004) 114–119.

[2] L. Aigner, P. Caroni, Depletion of 43-kD growth-associated protein in primary sensory neurons leads to diminished formation and spreading of growth cones, J. Cell Biol. 123 (1993) 417–429.

[3] S.A. Azizi, D. Stokes, B.J. Augelli, C. DiGirolamo, D.J. Prockop, Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats—similarities to astrocyte grafts, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 3908–3913.

[4] R.T. Ferri, P. Levitt, Regulation of regional differences in the differentiation of cerebral cortical neurons by EGF family-matrix interactions, Development 121 (1995) 1151–1160.

[5] C.P. Hofstetter, E.J. Schwarz, D. Hess, J. Widenfalk, A. El Manira, D.J. Prockop, L. Olson, Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 2199–2204.

[6] E.A. Jones, S.E. Kinsey, A. English, R.A. Jones, L. Straszynski, D.M. Meredith, A.F. Markham, A. Jack, P. Emery, D. McGonagle, Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells, Arthritis Rheum. 46 (2002) 3349–3360.

[7] G.C. Kopen, D.J. Prockop, D.G. Phinney, Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 10711–10716.

[8] H.G. Kuhn, H. Dickinson-Anson, F.H. Gage, Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation, J. Neurosci. 16 (1996) 2027–2033.
[9] Y. Li, J. Chen, L. Wang, L. Zhang, M. Lu, M. Chopp, Intracerebral transplantation of bone marrow stromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, Neurosci. Lett. 316 (2001) 67–70.
[10] Y. Li, M. Chopp, J. Chen, L. Wang, S.C. Gautam, Y.X. Xu, Z. Zhang, Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice, J. Cereb. Blood Flow Metab. 20 (2000) 1311–1319.
[11] A. Mahmood, D. Lu, L. Wang, Y. Li, M. Lu, M. Chopp, Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells, Neurosurgery 49 (2001) 1196–1203, discussion 1203-4.
[12] D.C. Marchion, E. Bicaku, A.I. Daud, D.M. Sullivan, P.N. Munster, Valproic acid alters chromatin structure by regulation of chromatin modulation proteins, Cancer Res. 65 (2005) 3815–3822.
[13] J.J. Minguell, A. Erices, P. Conget, Mesenchymal stem cells, Exp. Biol. Med. (Maywood) 226 (2001) 507–520.
[14] S.J. Peter, C.R. Liang, D.J. Kim, M.S. Widmer, A.G. Mikos, Osteoblastic phenotype of rat marrow stromal cells cultured in the presence of dexamethasone, beta-glycerolphosphate, and L-ascorbic acid, J. Cell Biochem. 71 (1998) 55–62.
[15] J. Priller, D.A. Persons, F.F. Klett, G. Kempermann, G.W. Kreutzberg, U. Dirnagl, Neogenesis of cerebellar Purkinje neurons from gene-marked bone marrow cells in vivo, J. Cell Biol. 155 (2001) 733–738.
[16] M. Saito, H. Sakagami, S. Fujisawa, Cytotoxicity and apoptosis induction by butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), Anticancer Res. 23 (2003) 4693–4701.
[17] J. Sanchez-Ramos, S. Song, F. Cardozo-Pelaez, C. Hazzi, T. Stedeford, A. Willing, T.B. Freeman, S. Saporta, W. Janssen, N. Patel, D.R. Cooper, P.R. Sanberg, Adult bone marrow stromal cells differentiate into neural cells in vitro, Exp. Neurol. 164 (2000) 247–256.
[18] J.R. Sanchez-Ramos, Neural cells derived from adult bone marrow and umbilical cord blood, J. Neurosci. Res. 69 (2002) 880–893.
[19] L. Santa Maria, C.V. Rojas, J.J. Minguell, Signals from damaged but not undamaged skeletal muscle induce myogenic differentiation of rat bone-marrow-derived mesenchymal stem cells, Exp. Cell Res. 300 (2004) 418–426.
[20] H. Song, C.F. Stevens, F.H. Gage, Astroglia induce neurogenesis from adult neural stem cells, Nature 417 (2002) 39–44.
[21] T. Wehner, M. Bontert, I. Eyupoglu, K. Prass, M. Prinz, F.F. Klett, M. Heinze, I. Bechmann, R. Nitsch, F. Kirchhoff, H. Kettenmann, U. Dirnagl, J. Priller, Bone marrow-derived cells expressing green fluorescent protein under the control of the glial fibrillary acidic protein promoter do not differentiate into astrocytes in vitro and in vivo, J. Neurosci. 23 (2003) 5004–5011.
[22] L.R. Zhao, W.M. Duan, M. Reyes, C.D. Keene, C.M. Verfaillie, W.C. Low, Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats, Exp. Neurol. 174 (2002) 11–20.