Experimental Neurology 233 (2012) 472–480

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Regular Article

The functional deficiency of bone marrow mesenchymal stromal cells in ALS patients is proportional to disease progression rate

Seong-Ho Koh [a,1], Wonki Baik [a,1], Min Young Noh [a], Goang Won Cho [a], Hyun Young Kim [a], Kyung Suk Kim [b], Seung Hyun Kim [a,*]

[a] Department of Neurology, Hanyang University College of Medicine, Seoul, Republic of Korea
[b] Bioengineering Institute, CoreStem Inc., Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 15 September 2011
Accepted 10 November 2011
Available online 19 November 2011

Keywords:
Amyotrophic lateral sclerosis
Prognostic factors
Neurotrophic factors
Mesenchymal stromal cells

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is caused by motor neuron death. The relationship between the prognosis of ALS patients and the function of their bone marrow mesenchymal stromal cells (BM-MSCs) is unclear. We designed this study to assess the correlation between the progression rate of the ALS Functional Rating Scale-revised version (ΔFS), which is reported to predict prognosis, and the pluripotency and trophic factor secreting capacity of ALS patients' BM-MSCs. We evaluated ΔFS in 23 ALS patients and isolated BM-MSCs from those patients and five healthy people. Levels of *Nanog*, *Oct-4*, and *Nestin* mRNA were examined to evaluate pluripotency, and levels of *BDNF*, *ECGF1*, *bFGF-2*, *HGF*, *IGF-1*, *PGF*, *TGF-1β*, *SDF-1α*, *GDNF*, *VEGF*, and *ANG* mRNA were examined to assess trophic factor secreting capacity. In addition, we measured the protein levels of Nanog, Oct-4, Nestin, SDF-1α, ANG, bFGF-2, VEGF, IGF-1, GDNF, and BDNF. mRNA levels of *Nanog*, *Oct-4*, *ECGF1*, *bFGF-2*, *HGF*, *IGF-1*, *PGF*, *TGF-1β*, *SDF-1α*, *GDNF*, *VEGF*, and *ANG* were negatively correlations with ΔFS. However, those of *Nestin* and *BDNF* were not significantly correlated with ΔFS. Similarly, Nanog, Oct-4, SDF-1α, ANG, bFGF-2, VEGF, IGF-1, and GDNF protein levels had a significant negative correlation with ΔFS. Results indicate that the pluripotency and trophic factor secreting capacity of the BM-MSCs of ALS patients are reduced in proportion to a poorer prognosis. We therefore suggest that healthy allogeneic BM-MSCs might be a better option for cell therapy in ALS patients.

Introduction

Amyotrophic lateral sclerosis (ALS) is one of the most common fatal neurodegenerative disorders. It is characterized by progressive motor neuron death in the spinal cord, brainstem, and motor cortex (Cleveland and Rothstein, 2001). In spite of numerous studies, the cause of ALS remains largely unknown and there is no cure. In addition, the factors affecting prognosis have not yet been completely established, although recent studies have shown that the ALS Functional Rating Scale-revised version (ALSFRS-r), its ratio, and its progression rate (ΔFS), are helpful in predicting patient survival time and prognosis (Kaufmann et al., 2005; Kimura et al., 2006; Kollewe et al., 2008).

Bone marrow mesenchymal stromal cells (BM-MSCs) have the capacity to self-renew and to differentiate into diverse cell types, including neuron-like cells (Caplan, 2007; Pittenger et al., 1999; Woodbury et al., 2000). They also secrete a variety of cytokines and growth factors (Caplan and Dennis, 2006; Koh et al., 2009; Ninichuk et al., 2006; Zhang et al., 2007). Based on these capacities of BM-MSCs, there have been many clinical trials using BM-MSCs for the treatment of ALS. Karussis et al. reported that intrathecal injection of autologous BM-MSCs is a safe and feasible procedure (Karussis et al., 2010). Mazzini et al. showed that BM-MSC implantation into the spinal cord of ALS patients was safe and that BM-MSCs could be useful for future ALS cell-based clinical trials (Mazzini et al., 2010). Our previous study also indicated that intrathecal injection of BM-MSCs is safe and has the potential for use as a disease modifying modality (Kim et al., 2009). Contrary to these results, however, Gamez et al. insisted that transplantation of BM-MSCs did not halt the course of ALS (Gamez et al., 2010).

All of these earlier investigations of the effect of autologous BM-MSC therapy in ALS patients led us to hypothesize that the functional capacity of BM-MSCs differs depending on the condition of patients. In this study, we examined whether the pluripotency and trophic factor secreting capacity of the BM-MSCs of ALS patients decrease in proportion to ΔFS as a measure of prognosis. Our aim was to assess whether autologous or allogeneic BM-MSCs are likely to be more useful in cell therapy in terms of their neurotrophic effects.

* Corresponding author at: Department of Neurology, College of Medicine, Hanyang University, #17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, Republic of Korea. Fax: +82 2 2296 8370.
E-mail address: kimsh1@hanyang.ac.kr (S.H. Kim).
[1] Both authors contributed equally to this work.

0014-4886/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2011.11.021

Materials and methods

Isolation and culture of MSCs

Ethical approval for this study was obtained from Hanyang University Hospital in Seoul, Korea (HYU-IRB-2004-643 and HYU-IRB-2005-452). The BM-MSCs used were left over from a human trial evaluating the effect of autologous BM-MSC transplantation in ALS patients (Kim et al., 2009). They were obtained from 23 ALS patients (A-MSC) and 5 age-matched healthy donor controls (H-MSC) (Table 1). A-MSCs were collected from probable and definite ALS patients who would undergo autologous MSC therapy. Diagnosis was based on El Escorial World Federation of Neurology criteria. Consenting patients that fulfilled the following three criteria were included in this study: (1) satisfying the El Escorial criteria for probable and definite sporadic ALS; (2) male or non-pregnant female aged 25 to 65 years; and (3) an ALSFRS-r scale of disease severity higher than 20 points at the time of inclusion. We excluded ALS patients with (1) high protein levels or lymphocytosis in the cerebrospinal fluid; (2) positive test results for anti-GM1 antibodies; (3) significant conduction blocks or slow conduction velocities (a reduction of <30%) in nerve conduction studies; (4) significant cardiac, renal, or hepatic failure or any other disease that may interfere with the ability to interpret the results of the study; (5) an active infection; and (6) cognitive decline or the inability to understand and sign the informed consent form.

Patients were injected twice with MSCs intrathecally (L2–3 level) at an interval of 1 month. Patients were clinically assessed based on the ALSFRS-r. The residual A-MSCs and H-MSCs post-transplantation were used for this study. All individuals provided written informed consent and understood the issues involved in the use of BM-MSCs for basic research. To obtain A-MSCs and H-MSCs, mononuclear cells were isolated by bone marrow aspiration at the iliac crest, followed by enrichment on a density gradient (Histopaque, density 1.077 g/ml; Sigma-Aldrich, St Louis, MO, USA) and two washes with Dulbecco's Modified Eagle's Medium containing low glucose (DMEM-LG; GIBCO BRL, Grand Island, NY, USA). Cells were cultured at a density of 2×10^5 cells/cm^2 in DMEM-LG with 10% fetal bovine serum (Hyclone, Waltham, MA, USA) at 37 °C in 5% CO_2 for 3 days. After removing non-adherent cells, the culture medium was changed twice per week. For passage, cells were detached with 0.25% trypsin/ethylene diamine tetraacetic acid for 3 min at 37 °C, seeded at a density of 4×10^3 cells/cm^2, and expanded to 80–90% confluence. MSCs were used at their fourth passage. Cell immunophenotype was confirmed by flow cytometry.

Fluorescence-activated cell sorting (FACS) analysis

To determine the mesenchymal phenotypes (Kobune et al., 2003) of the H- and A-MSCs, we used monoclonal antibodies against HLA-DR, CD49c, CD73, and CD105 (Abcam, Cambridge, MA, USA), CD34 (Santa Cruz Biotech, Santa Cruz, CA, USA), and CD45, CD29, CD90, and CD44 (Sigma-Aldrich). Briefly, MSCs at their fourth passage were stained in phosphate-buffered saline (PBS; Ca^{2+} and Mg^{2+}-free) supplemented with 5% fetal bovine serum (Hyclone). After the final wash, cells were fixed in 1% paraformaldehyde prior to analysis with a FACscan (Becton-Dickinson, USA) using FITC (fluorescein isothiocyanate)- or PE (phycoerythrin)-labeled goat-anti-mouse immunoglobulin as the isotype control. To account for non-specific binding, the same fluorochrome/protein ratio was used for the isotype control.

Real-time reverse transcription-polymerase chain reaction (RT-PCR)

To quantify gene expression, fourth passage H- and A-MSCs were cultivated under identical conditions and harvested at 80% confluence (usually after 2 days). Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and 3–5 μg were reverse-transcribed using RevertAidTM M-MuLV reverse transcriptase (MBI

Table 1
Demographic and clinical characteristics of ALS patients.

	Sex	Age of onset (years)	Duration to admission from 1st symptoms (months)	Age of admission (years)	Score of ALSFRSr at BM aspiration	1st symptom	Initial EEC	FVC	ΔFS	Riluzole	Familial ALS
A1	M	44	24	46	44	Limb onset	Probable	82	0.167	0	–
A2	M	35	25	37	43	Limb onset	Probable	81	0.200	0	–
A3	F	58	16	59	38	Limb onset	Probable	89	0.625	0	–
A4	F	40	4	40	43	Limb onset	Probable	99	1.000	0	–
A5	M	63	35	66	42	Limb onset	Probable	73	0.187	0	–
A6	M	36	8	37	45	Limb onset	Probable	63	0.375	0	–
A7	F	64	15	65	46	Limb onset	Probable	80	0.133	0	–
A8	M	57	10	58	40	Bulbar onset	Probable	61	0.800	0	–
A9	M	52	11	53	44	Limb onset	Probable	97	0.207	0	–
A10	F	56	4	57	46	Bulbar onset	Clinical probable, lab-supported	51	0.500	0	–
A11	F	58	27	60	27	Limb onset	Probable	63	0.780	0	–
A12	M	42	39	46	41	Limb onset	Probable	92	0.188	0	–
A13	F	38	73	44	35	Limb onset	Probable	73	0.115	0	–
A14	M	40	57	45	32	Limb onset	Probable	61	0.319	0	–
A15	F	49	52	53	38	Limb onset	Definite clinical	103	0.297	0	–
A16	M	49	34	52	32	Limb onset	Probable, labsupported	52	0.294	0	–
A17	M	34	14	36	43	Limb onset	Definite	80	0.417	0	–
A18	M	43	32	46	42	Limb onset	Probable	84	0.190	0	–
A19	F	36	26	38	40	Limb onset	Probable clinical	107	0.300	0	–
A20	M	50	29	53	28	Limb onset	Probable, lab-supported	85	0.350	0	–
A21	M	47	16	48	26	Limb onset	Probable	90	0.867	0	–
A22	F	38	46	42	39	Limb onset	Definite	75	0.194	0	–
A23	F	43	18	45	28	Limb onset	Probable	68	1.286	0	–
H1	M	–	–	39	–	–	–	–	0	–	–
H2	F	–	–	52	–	–	–	–	0	–	–
H3	M	–	–	49	–	–	–	–	0	–	–
H4	M	–	–	37	–	–	–	–	0	–	–
H5	F	–	–	47	–	–	–	–	0	–	–

1st symptom means the first symptom recognized by the patient; initial EEC, the initial diagnostic state of the patient based on El Escorial Criteria; FVC, forced vital capacity; and ΔFS, progression rate which is determined based on patient's subjective complaints and information confirmed by family members. A means ALS patient and H healthy control.