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Effect of Transplantation of Bone Marrow-Derived Mesenchymal Stem Cells on Mice Infected with Prions▽†

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Bone marrow-derived mesenchymal stem cells (MSCs) have been reported to migrate to brain lesions in experimental models of ischemia, tumors, and neurodegenerative diseases and to ameliorate functional deficits. In this study, we attempted to evaluate the therapeutic potential of MSCs for treating prion diseases. Immortalized human MSCs (hMSCs) that express the *LacZ* gene were transplanted into the unilateral hippocampi or thalami of mice, and their distributions were monitored by the expression of β-galactosidase. In mice infected with prions, hMSCs transplanted at 120 days postinoculation (dpi) were detected on the contralateral side at 2 days after transplantation and existed there even at 3 weeks after transplantation. In contrast, few hMSCs were detected on the contralateral side for mock-infected mice. Interestingly, the migration of hMSCs appeared to correlate with the severity of neuropathological lesions, including disease-specific prion protein deposition. The hMSCs also migrated to a prion-specific lesion in the brain, even when intravenously injected. Although the effects were modest, intrahippocampal and intravenous transplantation of hMSCs prolonged the survival of mice infected with prions. A subpopulation of hMSCs in the brains of prion-infected mice produced various trophic factors and differentiated into cells of neuronal and glial lineages. These results suggest that MSCs have promise as a cellular vehicle for the delivery of therapeutic genes to brain lesions associated with prion diseases and, furthermore, that they may help to regenerate neuronal tissues damaged by prion propagation.

Prion diseases are fatal neurodegenerative disorders of humans and animals that are strongly associated with the conversion of normal prion protein (PrP^{C}) to a disease-specific isoform of prion protein (PrP^{Sc}). Many inhibitors of PrP^{Sc} formation, investigated by using cells persistently infected with prions or an in vitro conversion reaction, have been reported as candidates for therapeutics (55). Several compounds or active/passive immunization with PrP showed a prophylactic effect when administered before, simultaneously with, or just after inoculation with prions (14, 19, 43, 50, 52). However, only a few compounds, such as amphotericin B and its derivative pentosan polysulfate, porphyrin derivatives, and certain amyloidophilic compounds, have been shown to be effective at prolonging survival when administered in the middle or late stage of prion infection (10, 13, 25, 27). In clinical trials, pentosan polysulfate seems to extend the survival of several patients beyond the mean but appears unable to arrest the progression of the disease (4, 45).

Recently, we demonstrated that intraventricular infusion of an anti-PrP monoclonal antibody (MAb) could antagonize disease progression even when initiated after clinical onset, although the distribution of the MAb was largely restricted to the hippocampus and thalamus (53). Thus, improved delivery of the MAb may enhance its beneficial effects. Additionally, because antagonizing PrP^{Sc} formation is not sufficient to restore degenerated lesions, it is necessary to pursue ways to regenerate degenerated neuronal tissues.

Bone marrow-derived mesenchymal stem cells (MSCs) are multipotent adult stem cells of mesodermal origin. They can differentiate into mesenchymal lineages, such as osteoblasts, adipocytes, and myocytes (15, 41, 44). Remarkably, they also *trans*-differentiate into nonmesodermal cell types, including neuronal and glial lineages (48, 61). A number of studies have shown that MSCs migrate to damaged neuronal tissues following cerebral or systemic transplantation in animal models of ischemia (2, 7), spinal cord injury (23), brain tumors (37), Parkinson's diseases (21, 30), and Niemann-Pick disease (24). The introduction of MSCs in these model contexts resulted in functional recovery; however, the precise mechanisms for restoration remain to be elucidated (36, 38).

In this study, we investigated the therapeutic potential of MSCs for prion diseases. Although the use of mouse MSCs is suitable for studying the effect of MSCs on mice infected with prions, we used immortalized human MSCs (hMSCs) here because of the lack of appropriate methods for the isolation of mouse MSCs at the beginning of the study. In addition, hMSCs can be readily expanded in cell culture; their phenotypes remain similar to those of the primary human MSCs (26); and

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hMSCs are reported to avoid allogeneic rejection when they are transplanted into rat brains in a model of ischemia (38). Here we show that the hMSCs can migrate to neuropathological lesions in prion-infected mice and that their transplantation prolongs the survival of such mice. In addition, we also show that hMSCs that have migrated to prion-specific lesions secrete trophic factors and differentiate into cells of neuronal and glial lineages.

MATERIALS AND METHODS

MSCs. A retroviral vector, Rx-LacZ-bsr, containing the expression units of the *lacZ* gene and a gene conferring blasticidin resistance, were generated as described elsewhere (63). The recombinant retrovirus was used to transfect human bone marrow-derived MSCs that had been immortalized with the human telomerase catalytic subunit gene (26), and hMSCs were selected in the presence of 10 μg/ml of blasticidin. hMSCs stably expressing β-galactosidase (β-Gal) were cultured with Dulbecco's modified Eagle medium (DMEM) (Sigma Chemical Co., St. Louis, MO) containing 10% fetal bovine serum under a humidified atmosphere of 5% CO_2 at 37°C.

Mice and prion inoculation. Animal experiments were performed according to protocols approved by the Institutional Committee for Animal Experiments. Four-week-old female Jcl:ICR mice were purchased from CLEA Japan, and all mice were acclimatized for a week prior to use. Mice were intracerebrally inoculated with 20 μl of a 10% (wt/vol) brain homogenate from Jcl:ICR mice infected with the scrapie strain Obihiro or Chandler. Mice assigned to the mock-infected group were intracerebrally inoculated with 20 μl of a 10% (wt/vol) brain homogenate from age-matched uninfected Jcl:ICR mice. All mice were maintained on ad libitum feed and water with a 12-h light/dark cycle.

Transplantation of hMSCs. For transplantation of cells into the hippocampus or thalamus, mice were anesthetized by intramuscular injection of xylazine (10 mg/kg) and ketamine (50 mg/kg) and were placed on a stereotaxic apparatus (Narishige, Japan). After a linear scalp incision, burr holes were drilled to accommodate stereotaxic placement into the left hippocampus (2.0 mm caudal and 2.1 mm lateral to the bregma; depth, 2 mm) or thalamus (2.0 mm caudal and 1.5 mm lateral to the bregma; depth, 3.2 mm). hMSCs (1×10^5 cells in 2 μl phosphate-buffered saline [PBS]) were transplanted over a period of 15 min using a Hamilton syringe with a 31-gauge needle set in a micromanipulator. For transplantation of hMSCs via a peripheral route, 1×10^6 hMSCs were injected intravenously through the tail vein.

Immunohistochemistry. Mouse brains were frozen in Tissue-Tek OCT compound (Sakura, Japan), and cryosections (10 μm thick) were prepared as described elsewhere (53). Coronal sections were dried and fixed with ice-cold methanol for 15 min. A mouse anti-β-Gal MAb (catalog no. Z3783; Promega, Madison, WI) was conjugated with Alexa Fluor 488 by using a protein labeling kit (Molecular Probes, Eugene, OR) for the detection of hMSCs by direct staining. The following antibodies were used for the detection of various tropic factors: rabbit polyclonal antibodies against nerve growth factor (NGF) (Santa Cruz Biotechnology, Santa Cruz, CA), brain-derived neurotropic factor (BDNF) (Chemicon, Temecula, CA), neurotrophin 3 (NT3) (Chemicon), and neurotrophin 4/5 (NT4/5) (Santa Cruz Biotechnology); a rabbit MAb against vascular endothelial growth factor (VEGF) (clone EP1176Y; Abcam, Cambridge, MA); and a mouse MAb against ciliary neurotropic factor (CNTF) (clone A-11; Santa Cruz Biotechnology). As neuronal markers, we used a mouse MAb against microtubule-associated protein 2 (MAP2) (clone HM-2; Sigma Chemical Co.) for neurons, rabbit polyclonal antibodies (Dako, Denmark) against glial fibrillary acidic protein (GFAP) for astrocytes, and a mouse MAb against cyclic nucleotide phosphodiesterase (CNPase) (clone 11-5B; Chemicon) for oligodendrocytes. All sections were incubated with primary antibodies for 1 h at 37°C. To detect trophic factors and neural markers, the sections were subsequently incubated with an Alexa Fluor 546-conjugated anti-mouse antibody or an Alexa Fluor 555-conjugated anti-rabbit antibody (Molecular Probes) for 1 h at room temperature. After a wash with PBS, sections were mounted with Vectashield containing propidium iodide or 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA) and were examined with a Nikon C1 laser confocal microscope. To exclude the possibility of nonspecific reactions between the Alexa Fluor 546-conjugated anti-mouse antibody and mouse tissues, we carried out the immunostaining without primary antibodies and confirmed that the level of nonspecific binding of the Alexa Fluor 546-conjugated anti-mouse antibody was negligible.

For the detection of PrP^{Sc} accumulation and astrocytosis, mouse brains were fixed in 10% formalin and embedded in paraffin. Coronal sections (thickness, 4 μm) were subjected to hematoxylin-eosin (HE) staining or immunohistochemistry as described elsewhere (17, 53).

Proliferation assay. To detect proliferating cells in the brain, 50 mg of bromodeoxyuridine (BrdUrd; Sigma Chemical Co.) per kg of body weight in PBS with 0.007 M NaOH was administered to mice intraperitoneally twice a day for a week. BrdUrd administration was initiated soon or 2 weeks after the transplantation of hMSCs into the hippocampus. Mice were sacrificed 24 h after the last BrdUrd administration, and the brains of these mice were then prepared for cryosectioning. The sections were pretreated with 2 M HCl for 30 min at 37°C, followed by a neutralization step with 0.1 M borate buffer for 15 min at room temperature. BrdUrd in nuclei was detected using a fluorescein isothiocyanate-conjugated anti-BrdUrd MAb (clone B33.1; Abcam).

Cell migration assay. Prion- or mock-infected mice were sacrificed at 120 days postinoculation (dpi), and the brains of these mice were homogenized to 20% in DMEM (Sigma Chemical Co.). The homogenates were centrifuged at 10,000 × *g* for 10 min at 4°C, and the resulting supernatants were filtered (pore size, 0.22 μm). The brain extracts were aliquoted and stored at −80°C until use. Migration of hMSCs to brain extracts was analyzed using a QCM 24-well colorimetric cell migration assay kit (Chemicon). The hMSCs (approximately 80% confluent) were starved by incubation with serum-free medium 1 day before use. Then hMSCs were harvested, and a cell suspension (5×10^4 cells) was added to the insert well. The lower chambers were supplied with serum-free DMEM containing 1.0 or 0.1% brain extract. Twenty-four hours after incubation, hMSCs that had migrated through the polycarbonate membrane were extracted, and the absorbance at 560 nm was measured according to the manufacturer's instructions.

RESULTS

Distribution of hMSCs to the neuropathological lesions of prion disease. To test if hMSCs migrate to brain lesions caused by prion infection, we transplanted hMSCs into the left hippocampi of prion- or mock-infected mice at 120 dpi and monitored the distribution of β-Gal-positive hMSCs at 2 days and 1, 2, and 3 weeks after transplantation. In mock-infected mice, hMSCs were detected in the left hippocampus (transplanted side), but few hMSCs were detected in the contralateral hippocampus at 2 days to 3 weeks after transplantation (Fig. 1a). In contrast, hMSCs were detected both on the transplanted sides and on the contralateral sides of the hippocampi of mice infected with strain Chandler even 2 days after transplantation. Thereafter, hMSCs were constantly observed on both sides of the hippocampus during the observation period (every week after transplantation up to 3 weeks [Fig. 1a]). For each experimental group, we examined three mice at each time point and confirmed the similar results. One to 3 weeks after transplantation, hMSCs were also detected in the cortices, cerebella, medullae oblongatae (see Fig. 3b), and thalami (data not shown) of mice infected with prions, where intense PrP^{Sc} accumulations and astrocytosis were observed (see Fig. S1 in the supplemental material). Ramified hMSCs were observed in the corpus callosum; their morphologies differed from those observed in the contralateral hippocampus (Fig. 1b).

Transplantation of hMSCs into the left thalamus led to similar results. In mock-infected mice, hMSCs remained in the transplanted area, and few hMSCs migrated to the contralateral thalamus or to other regions. In contrast, many hMSCs were detected in the contralateral thalami (Fig. 1c) and hippocampi (data not shown) of mice infected with prions by 3 weeks posttransplantation.

We noticed a striking difference in the neuropathology of the hypothalami of mice infected with strain Obihiro versus strain Chandler. Specifically, PrP^{Sc} accumulations, astrocytosis, and spongiosis in the hypothalami of mice infected with

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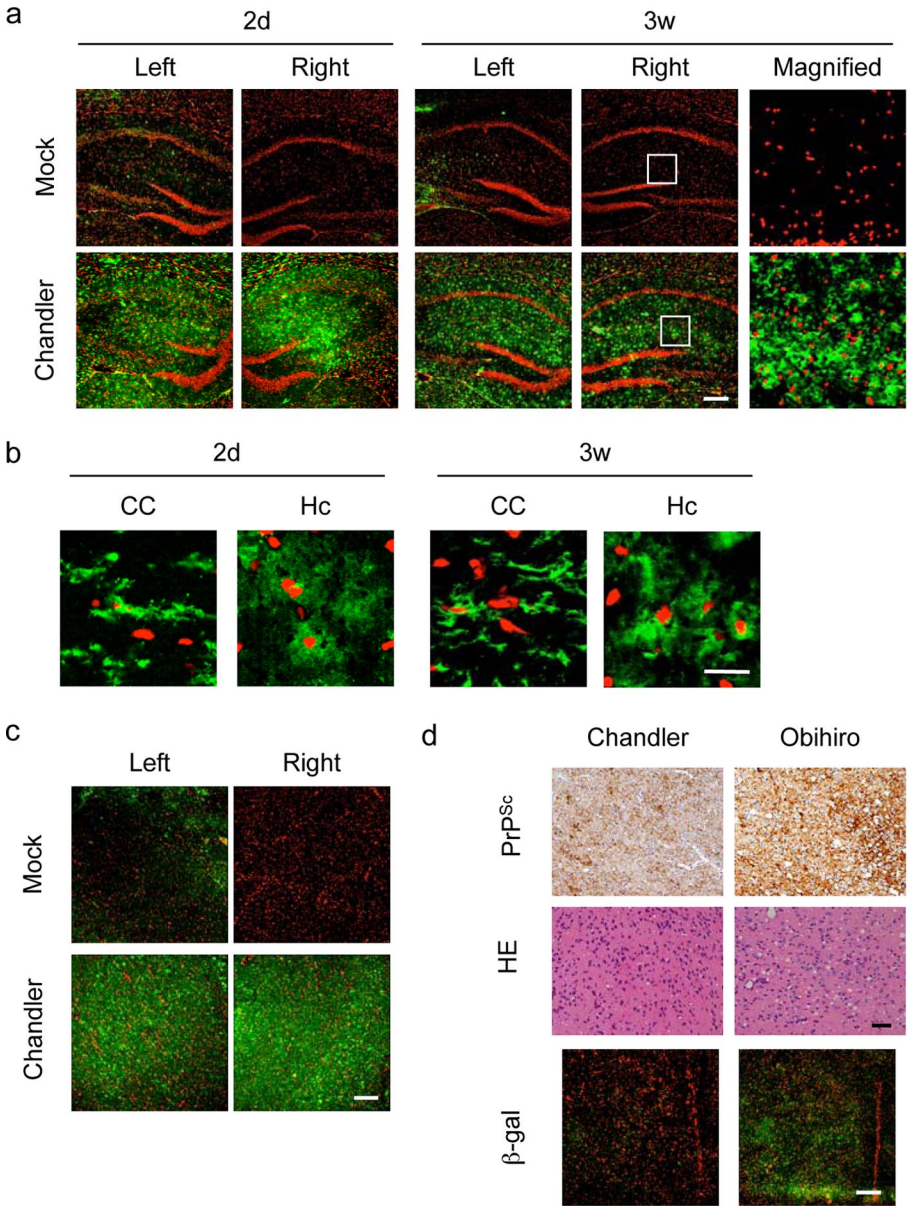

FIG. 1. Distribution of hMSCs to the neuropathological lesions of prion disease. At 120 dpi, hMSCs (1×10^5 cells) were transplanted into the left hippocampi or thalami of mice infected with strain Chandler and into those of age-matched mock-infected mice. Mice were sacrificed at 2 days or at 1, 2, or 3 weeks posttransplantation. Cryosections were stained with an Alexa Fluor 488-conjugated anti-β-Gal MAb (green) and counterstained with propidium iodide (red). (a) hMSCs in the hippocampus. Ipsilateral (left) and contralateral (right) hippocampi 2 days (2d) and 3 weeks (3w) posttransplantation are shown. The rightmost panels show magnified images of the regions boxed in the panels immediately to the left. Bar, 200 μm. (b) Morphology of hMSCs in the corpora callosa (CC) and contralateral hippocampi (Hc) of mice infected with strain Chandler at 2 days and 3 weeks posttransplantation. Bar, 20 μm. (c) hMSCs in the thalamus. Ipsilateral (left) and contralateral (right) thalami 3 weeks posttransplantation are shown. Bar, 200 μm. (d) Migration of hMSCs to the hypothalamus. (Top and center) Results of immunostaining for PrP^{Sc} and HE staining of the hypothalami of mice infected with strain Obihiro or Chandler are shown at 150 dpi. Bar, 100 μm. (Bottom) The hMSCs were detected in the hypothalamus (β-Gal) 3 weeks after transplantation into the left hippocampus. Bar, 200 μm.

strain Obihiro are more severe than those for mice infected with strain Chandler (Fig. 1d; see also Fig. S1 in the supplemental material). Consistent with the severity of neuropathological lesions, more hMSCs migrated to the hypothalami of mice infected with strain Obihiro than to those of mice infected with strain Chandler (Fig. 1d; β-Gal). These results suggest that hMSCs are capable of migrating to brain lesions caused by prion infection.

Migration of hMSCs in response to prion-specific lesions. To confirm the migration of hMSCs to lesions where PrP^{Sc} accumulates, we transplanted hMSCs into the left hippocampi of mice infected with strain Chandler at 73, 100, and 120 dpi, and we analyzed their migration to the contralateral (right) side a week after transplantation. When hMSCs were transplanted at 73 dpi, many hMSCs were detected on the transplanted side but fewer hMSCs were detected in the contralat-

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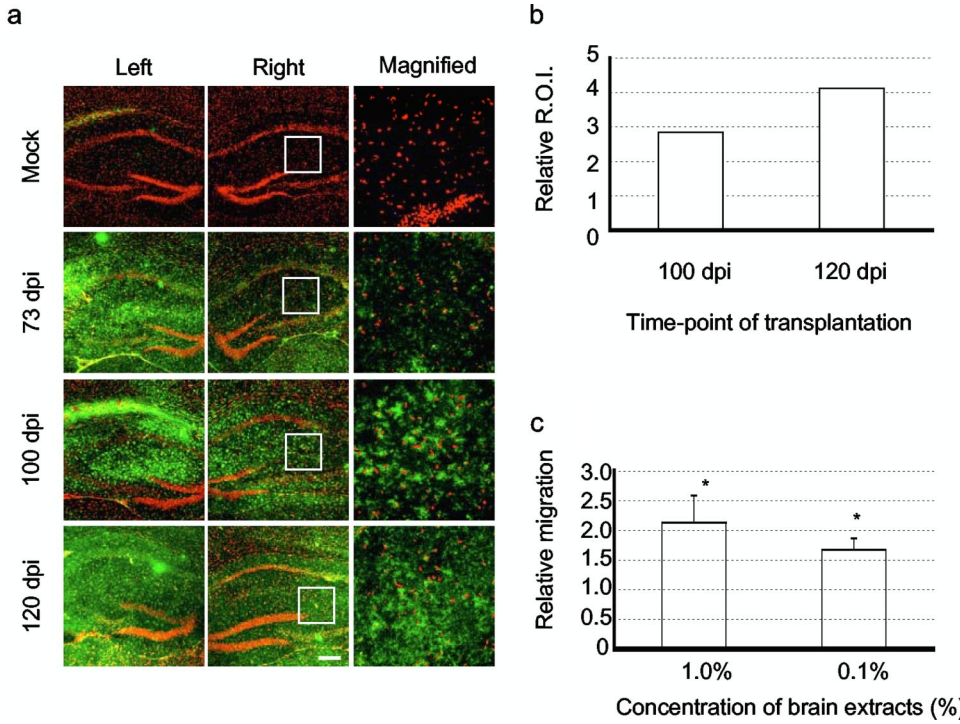

FIG. 2. Migration of hMSCs in response to prion-specific lesions. hMSCs (1×10^5 cells) were transplanted into the left hippocampi of mice infected with strain Chandler at 73, 100, or 120 dpi and into those of mock-infected mice at 120 dpi. One week after transplantation, the migration of hMSCs (green) to the contralateral hippocampus (right) was analyzed. The rightmost panels show magnified images of the regions boxed in the panels immediately to the left. Bar, 200 μm. (b) Quantification of hMSC migration. Areas positive for β-Gal in the contralateral hippocampus (regions of interest [ROI]) were measured using NIH Image J. The graph shows the levels of ROIs for mice transplanted at 100 and 120 dpi relative to that for a mouse transplanted at 73 dpi. (c) Migration of hMSCs to extracts from the brains of prion-infected mice. Insert wells containing an hMSC suspension were placed in the lower chambers, which contained 1.0 or 0.1% brain extract in serum-free DMEM, and were incubated for 24 h. The mean migration of hMSCs to brain extracts from mock-infected mice was arbitrarily set at 1, and the relative migration to brain extracts from mice infected with strain Chandler is indicated. Means and standard deviations from three independent assays (triplicate in each assay) are shown. *, $P < 0.05$.

eral hippocampus. In contrast, migration of hMSCs to the thalamus, where moderate PrP^{Sc} deposition had already occurred, was clearly observed (data not shown). In addition, more hMSCs were detected in the contralateral hippocampus when the transplantation was carried out at later time points (Fig. 2a). To compare the migration of hMSCs quantitatively, the total area of the hMSCs in the contralateral hippocampus (areas positive for β-Gal) was measured using the NIH Image J program. Compared to the migration of hMSCs to the contralateral hippocampus a week after the transplantation at 73 dpi, 2.8 and 4.1 times more hMSCs were detected when the transplantation was done at 100 and 120 dpi, respectively (Fig. 2b). We examined at least two mice from each experimental group and confirmed the consistency of the findings. Since PrP^{Sc} accumulation and astrocytosis in the hippocampi of mice infected with strain Chandler were first detected around 90 dpi and the levels of PrP^{Sc} accumulation and astrocytosis increased gradually thereafter (see Fig. S1 in the supplemental material), the migration of hMSCs to the contralateral hippocampus appeared to correlate with the severity of pathological changes.

Next, we analyzed the migration of hMSCs to brain extracts from prion-infected mice in vitro. Insert wells of a QCM 24-well colorimetric cell migration assay kit containing hMSCs were placed in the lower chambers, which contained 1.0 or 0.1% brain extract from prion- or mock-infected mice, and were incubated for 24 h. Cells that had migrated to the back sides (facing the lower chamber) of the membranes of the insert wells were quantified according to the supplier's instructions. Compared to the migration to brain extracts from mock-infected mice, approximately 2 and 1.5 times more hMSCs had migrated into the lower chambers containing 1.0% and 0.1% brain extracts from prion-infected mice, respectively (Fig. 2c). This suggests that chemoattractive factors that promote the migration of hMSCs are produced by the lesion caused by prion infection.

Migration of hMSCs into the brain after intravenous transplantation. MSCs have been reported to migrate to a site of brain injury even when they are introduced via intravenous injection (37, 38). To test if a similar phenomenon could be observed in prion-infected mice, hMSCs were intravenously inoculated into mice infected with strain Chandler or into mock-infected mice at 120 dpi. In mice infected with strain Chandler, hMSCs were observed in the hippocampus and thalamus even at 2 days after transplantation (data not shown). The cells showed a symmetrical distribution and appeared to increase in number in these tissues by 3 weeks posttransplantation (Fig. 3a; see also Fig. S2 in the supplemental material). In contrast, few MSCs were detected in the brains of mock-

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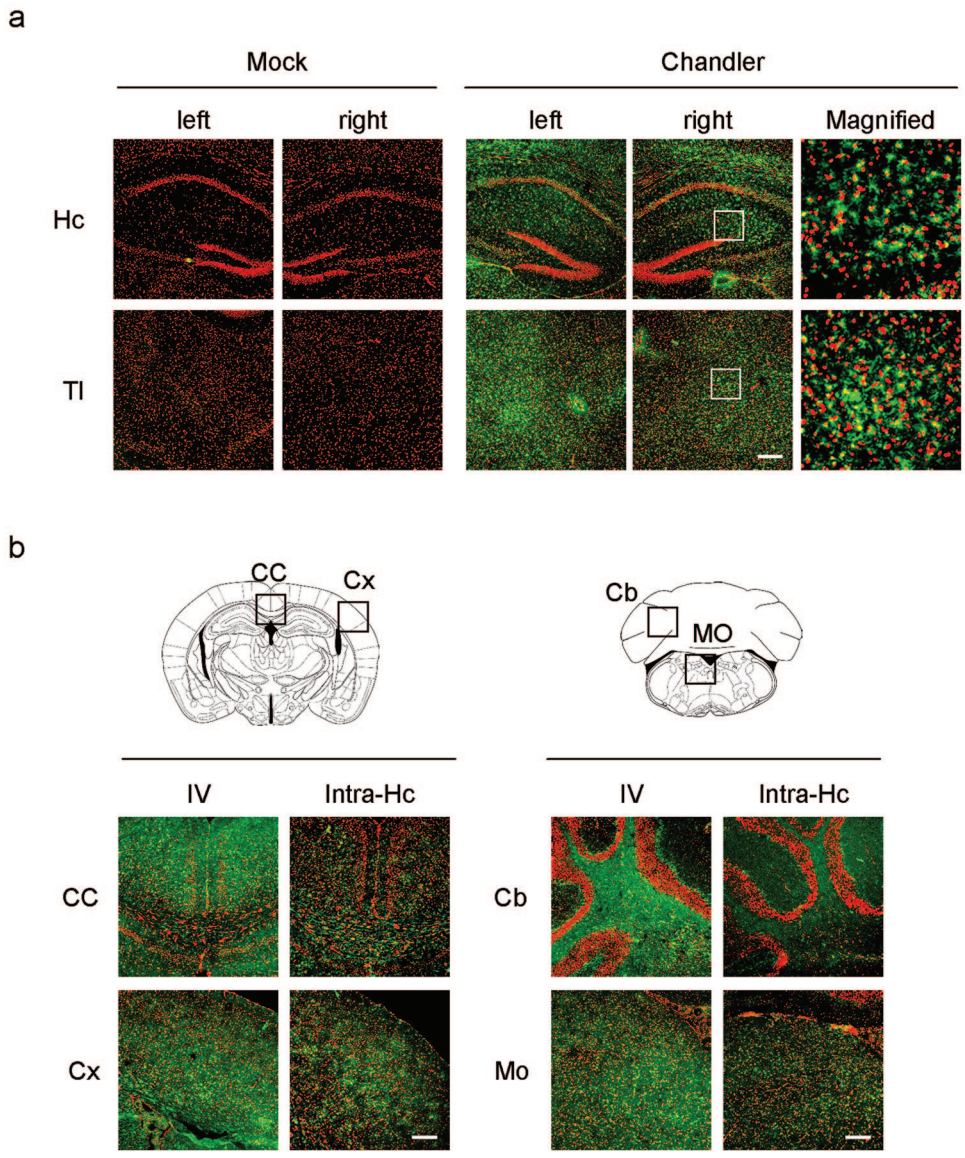

FIG. 3. Migration of hMSCs into the brain after intravenous transplantation. hMSCs (1×10^6 cells) were intravenously injected into mice infected with strain Chandler and into mock-infected mice at 120 dpi. Three weeks after injection, cryosections were prepared and stained with an anti-β-Gal MAb. (a) Presence of hMSCs in the left and right hippocampus (Hc) and thalamus (Tl) 3 weeks postinjection. The rightmost panels show magnified images of the regions boxed in the panels immediately to the left. Bar, 200 μm. (b) Distribution of hMSCs transplanted into the left hippocampus (Intra-Hc) or introduced via intravenous injection (IV). At 120 dpi, the hMSCs were transplanted into the left hippocampi (1×10^5 cells) or injected via the tail veins (1×10^6 cells) of mice infected with strain Chandler. The presence of hMSCs in the corpus callosum (CC), cortex (Cx), cerebellum (Cb), and medulla oblongata (MO) 3 weeks posttransplantation is shown. These brain regions are boxed on the images taken from Paxinos and Franklin and reprinted with permission of the publisher (39). Bars, 200 μm.

infected mice, demonstrating that the hMSCs migrated to the brain lesions caused by prion propagation. At each time point, we examined two mice for each experimental group and confirmed the consistency of the findings. The hMSCs were also well distributed in other brain regions, including the cerebral cortex, cerebellum, and medulla oblongata (Fig. 3b); however, consistent with the results shown in Fig. 3b, they did not migrate well to the hypothalamus (see Fig. S2 in the supplemental material). There was no difference in the area of hMSC distribution following intravenous versus intrahippocampal transplantation except at the corpus callosum. More hMSCs were observed in the corpus callosum after transplantation into the hippocampus than after intravenous injection, suggesting that cells migrate to the contralateral side through the corpus callosum after intrahippocampal transplantation (2).

Effects of transplantation of hMSCs on the survival of prion-infected mice. To examine whether the transplantation of hMSCs can ameliorate prion diseases, hMSCs were transplanted into the left hippocampi of mice infected with strain Chandler at 90 dpi. Figure 4 shows the survival curve for these mice. The intrahippocampal transplantation of hMSCs prolonged the survival of mice infected with strain Chandler

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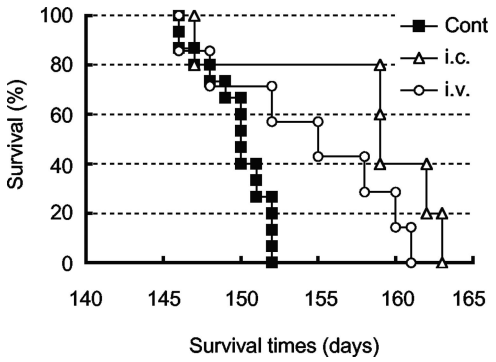

FIG. 4. Prolongation of survival of prion-infected mice by transplantation of hMSCs. For intracerebral (i.c.) transplantation, hMSCs (1×10^5 cells) were transplanted into the left hippocampi of mice infected with strain Chandler at 90 dpi ($n = 5$). For intravenous (i.v.) transplantation, 1×10^6 hMSCs were injected via the tail vein at 120 dpi ($n = 7$). The hMSC-transplanted and nontransplanted control (Cont) ($n = 15$) mice were observed until they reached the terminal stage of the disease. The graph shows survival curves.

(158 ± 6 days; $n = 5$) over that of the nontransplanted control group (150 ± 2 days; $n = 15$). Thus, hMSC transplantation prolonged mean survival by only 8 days, but this difference was statistically significant ($P < 0.01$ by the log rank test). We also transplanted hMSCs via the tail veins of mice infected with strain Chandler at 120 dpi. The transplantation of hMSCs via this peripheral route appeared not to be effective for nearly half of the mice; however, the remaining mice survived beyond the mean survival of the nontransplanted control group. Although the mean survival of hMSC-transplanted mice (154 ± 6 days; $n = 7$) was only a little longer than that of the control group, this difference was also significant ($P < 0.05$ by the log rank test). The fact that survival time was prolonged even when hMSCs were transplanted via a peripheral route after clinical onset (120 dpi) suggests that hMSCs have therapeutic potential for prion diseases. Since both intracerebral and intravenous transplantation of hMSCs prolonged the survival of prion-infected mice, we further analyzed the transplanted hMSCs.

Proliferation of hMSCs after transplantation. To examine the proliferation state of hMSCs that had migrated to lesions, BrdUrd was systemically administered after the transplantation of hMSCs to the left hippocampus. Three weeks posttransplantation, many BrdUrd-labeled nuclei were detected in the contralateral hippocampi and thalami of mice infected with strain Chandler (see Fig. S3 in the supplemental material), where many hMSCs had migrated (Fig. 1a). In contrast, few BrdUrd-labeled nuclei were detected in the contralateral hippocampi and thalami of mock-infected mice (see Fig. S3 in the supplemental material), although a few cells with BrdUrd-labeled nuclei were detected on the transplanted side (data not shown). BrdUrd-labeled cells were also observed in the cerebella and medullae oblongatae of mice infected with strain Chandler (data not shown). We examined two mice for each experimental group and confirmed the similar results. These results suggest that transplanted hMSCs are capable of proliferating in the microenvironment caused by prion propagation.

Expression of trophic factors in hMSCs. It is known that MSCs migrate to a site of injury in the brain and produce various trophic factors (8, 29). To ask if something similar happens in the case of prion disease, we next assayed the production of trophic factors in our model system. hMSCs were transplanted into the left thalamus at 120 dpi, and one mouse in each group was sacrificed and examined for the production of human trophic factors at 2 days and 1 and 3 weeks after transplantation. Immunoreactivities for human BDNF, NT3, and VEGF in the ipsilateral thalami of mice infected with strain Chandler became more intense from 2 days to 3 weeks posttransplantation. In contrast, no obvious increases, but rather decreases, in the signals of these trophic factors were observed for mock-infected mice (Fig. 5). Additionally, the expression of NGF, NT4/5, and CNTF was also upregulated (data not shown). These results suggest that hMSCs produce a variety of trophic factors in response to the neurodegeneration caused by prion infection.

Interestingly, only subpopulations of the hMSCs appeared to be positive for NT3 and BDNF. In addition, parts of the regions positive for these factors did not overlap with β-Gal staining. Since these antibodies are specific to human trophic factors and will not react with the corresponding mouse trophic factors, the presence of NT3 and BDNF in areas negative for β-Gal may represent trophic factors secreted from hMSCs and bound to mouse brain cells.

Differentiation of hMSCs. MSCs are known to differentiate into cells of neuronal and glial lineages in vivo and in vitro (11, 48, 61). We next asked if hMSCs differentiate into neuronal and glial cells in response to the lesions of prion diseases. At 3 weeks after transplantation into the thalamus, hMSCs positive for the neurodifferentiation marker MAP2, GFAP, or CNPase were detected in the brains of mice infected with strain Chandler (Fig. 6), although a relatively small number of hMSCs were positive for each marker. In contrast, no hMSCs positive for MAP2, GFAP, or CNPase were observed in the brains of mock-infected mice (data not shown), suggesting that neuronal and glial differentiation of hMSCs occurs in response to the neurodegeneration caused by prion infection. The GFAP-positive hMSCs were detected in the hippocampus, thalamus, and medulla oblongata. In contrast, MAP2-positive hMSCs were detected primarily in the hippocampus, cortex, and cerebellum, and CNPase-positive hMSCs were detected mainly in the cortex (data not shown).

DISCUSSION

The primary purpose of this study was to evaluate the potential of MSCs for treating prion diseases in a mouse model. For this purpose, the use of mouse MSCs would have been desirable; however, no appropriate method for the isolation of mouse MSCs from bone marrow had been established by the beginning of this study. On the other hand, it was well known that MSCs avoid allogeneic rejection (47). Thus, we adopted hMSCs to the mouse model and showed that hMSCs responded to the neuropathological lesions of prion diseases and may have therapeutic potential. Transplantation of MSCs is known to ameliorate neurological dysfunctions in experimental models (7, 23, 24, 30, 37). In clinical trials in which autologous MSCs are transplanted into patients with multiple system atrophy (28) or amyotrophic lateral sclerosis (33), or into patients who have suffered a stroke (3), there is some evidence of

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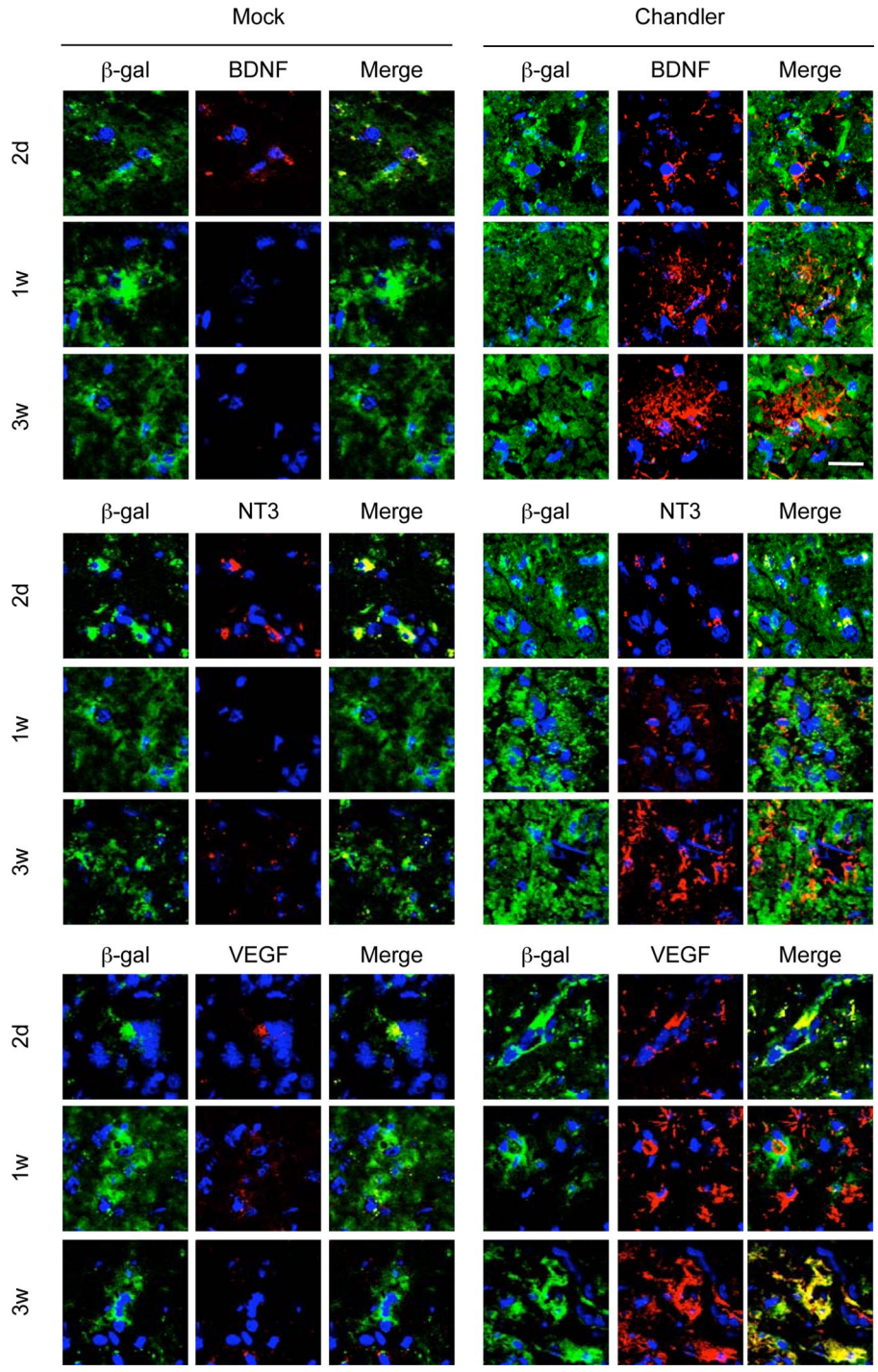

FIG. 5. Expression of trophic factors in hMSCs. hMSCs (1×10^5 cells) were transplanted into the left thalami of mice infected with strain Chandler and into those of mock-infected mice at 120 dpi. Two days (2d), 1 week (1w), and 3 weeks (3w) posttransplantation, cryosections were prepared and doubly stained with an anti-β-Gal MAb, for hMSCs (green), and an antibody against a human trophic factor (BDNF, NT3, or VEGF) (red). Nuclei were counterstained with DAPI (blue). Bar, 20 μm.

a beneficial effect without any adverse effects. How the introduction of MSCs leads to improved outcomes is not yet clear. However, the transplanted MSCs are known to migrate and home to a site of injury. Moreover, in this context, MSCs are expected to restore injured tissues by protecting neural tissues via secretion of various trophic factors (8), promotion of angiogenesis (20), stimulation of the proliferation and differentiation of endogenous neural stem cells (36), integration into tissues by differentiation or cell fusion (1), and modulation of the local immune response (64).

Here we showed that the level of migration of MSCs to the contralateral side of the mouse brain correlates with the de-

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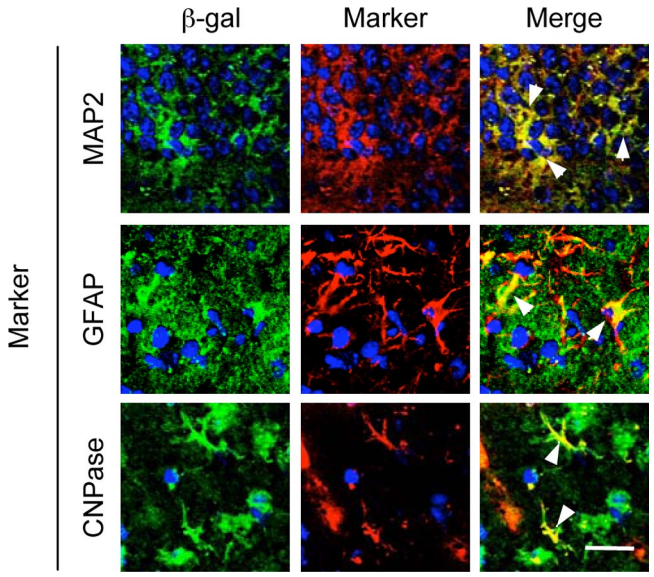

FIG. 6. Differentiation of hMSCs into cells of neuronal and glial lineages. At 120 dpi, hMSCs (1×10^5 cells) were transplanted into the left thalami of mice infected with strain Chandler. Three weeks post-transplantation, cryosections were prepared and doubly stained with an anti-β-Gal MAb, for hMSCs (green), and an antibody against a marker for neurons (MAP2), astrocytes (GFAP), or oligodendrocytes (CNPase) (red). Nuclei were counterstained with DAPI (blue). Arrows indicate hMSCs positive for each marker protein. The brain region for MAP2 and GFAP is the ipsilateral hippocampus, while that for CNPase is the cerebral cortex. Bar, 20 μm.

gree of PrP^{Sc} accumulation and the severity of histopathological changes in the prion-infected brain (Fig. 1d and 2). Although no antigen-specific humoral or cellular immune response is provoked in prion diseases, microglial activation and astrocytosis are prominent features of the diseases. Indeed, the expression of inflammatory cytokines and chemokines, which are likely to be produced in glial cells, is upregulated in the middle to late stage of prion infection (5, 6). In addition, brain extracts from prion-infected mice promoted chemotaxis of hMSCs in vitro. These results suggest that certain factors produced in the brains of prion-infected mice act as chemoattractive factors for hMSCs, although it is not clear whether the effects of mouse factors on human MSCs are as efficient as those on homologous MSCs. Monocyte chemoattractive protein 1 (known as CCL2), interleukin-8 (IL-8), and macrophage inflammatory protein 1α (known as CCL3) have been reported to enhance the migration of MSCs to ischemic brain tissue (57, 58). It was recently reported that an interaction between stromal cell-derived factor 1 (known as CXCL12), produced in ischemic brain lesions, and CXCR4, expressed on MSCs, plays an important role in the migration of MSCs (59). Because the inflammatory response and glial activation are common events in many neurological disorders, it is conceivable that a similar mechanism may account to some extent for the migration of MSCs to brain lesions associated with prion disease. For instance, we have found that the expression of CCL3 is upregulated in the thalami and medullae of prion-infected mice (unpublished observation). Experiments are under way to identify the chemoattractive factors by use of an in vitro chemotaxis assay.

MSCs have been reported to migrate to a site of brain injury even when intravenously injected (37, 38). Consistent with previous reports, our results showed here that hMSCs transplanted via intravenous injection travel to areas of brain lesions in prion-infected mice (Fig. 3). In prion diseases, although impairment of the blood-brain barrier (BBB) was observed in the cerebellum (56), no significant impairment of the BBB was observed in the hippocampus or cerebral cortex at the time of clinical onset or even at a later stage (42, 56). Thus, passive translocation of MSCs to the brain parenchyma through a disrupted BBB seems unlikely. Instead, active transendothelial migration of MSCs, similar to the recruitment of leukocytes and monocytes from the bloodstream to an inflammation site, is expected to be involved in the engraftment of MSCs transplanted via intravenous injection. Vascular cell adhesion molecule 1 and p-selectin expressed on the endothelium are important for the adhesion of MSCs to the endothelium via the β1 integrin VLA-4 (16, 46, 51). Proinflammatory cytokines, such as tumor necrosis factor alpha and IL-1β, upregulate the expression of adhesion molecules in endothelial cells (32). Indeed, tumor necrosis factor alpha and IL-1β are upregulated during the course of prion disease (6, 49), suggesting that these cytokines induce the adherence of MSCs to the endothelium and their subsequent transendothelial migration to the brain lesions. Understanding how the migration of MSCs to brain lesions affected by prion diseases is regulated, and further elucidation of the mechanisms underlying the tropism of MSCs, may provide new insight into the engraftment of MSCs as it relates to the progression and possible treatment of neurodegenerative diseases.

The ability of MSCs to migrate to a site of injury has been given particular attention, because it suggests that these cells can act as a vehicle for gene therapy in addition to aiding in the regeneration of degenerated tissues. Indeed, MSCs expressing genes of therapeutic potential showed a greater positive effect on functional recovery than unmodified MSCs (24, 37, 38). Transgenic expression of anti-PrP antibodies (22), a fusion protein between PrP^C and the Fc portion of immunoglobulin (PrP-Fc) (34), and dominant-negative PrP mutants (40) inhibited prion propagation. In addition, expression of anti-PrP Fab fragments and PrP-Fc in the brain by virus vectors has been reported to antagonize prion propagation in the brain (18, 62). Furthermore, intraventricular infusion of an anti-PrP MAb slowed the formation of neuropathological lesions and prolonged the survival of prion-infected mice even when the MAb was administered at clinical onset (53). However, large macromolecules, such as immunoglobulins, are expected to be delivered to the lesions inefficiently. Indeed, the distribution of MAbs was restricted primarily to the hippocampus and thalamus, even when the MAbs were infused directly into the lateral ventricle (53). Therefore, the observation that hMSCs target and home to brain lesions associated with prion diseases indicates the potential utility of hMSCs as a cellular vehicle for the delivery of therapeutic genes to brain lesions.

We showed here that microenvironments in the brain lesions associated with prion disease stimulate MSCs to produce various trophic factors: BDNF, NGF, VEGF, and others. These trophic factors are reported to have antiapoptotic effects, to promote nerve fiber regeneration, and to induce endogenous cell proliferation and angiogenesis in injured brains (9, 29, 31).

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It remains to be elucidated whether the prolonged survival of prion-infected mice by hMSC transplantation can be attributed to the secretion of trophic factors from hMSCs. Although hMSCs alone may have the ameliorative effect to some extent, they could not arrest the disease progression caused by prion propagation. Similarly, it has been shown that antagonizing prion propagation can slow disease progression but cannot ameliorate functional deficits (13, 25, 53). Thus, it seems possible that the combination of MSCs with inhibitors of prion propagation would have a synergistic effect in the treatment of prion diseases.

Replacement of damaged neurons with differentiated MSCs or their fusion with MSCs after MSC transplantation is an attractive possible route to the restoration of neurological functions (11, 54, 60). In this study, we showed that small populations of MSCs were differentiated into cells expressing neuronal, astrocyte, or oligodendrocyte markers. Because only a small portion of transplanted MSCs differentiated into a neuronal and a glial lineage in vivo, it seems unlikely that the prolongation of survival could be attributed directly to differentiation. However, induction of neuronal differentiation in vitro prior to transplantation improves functional outcomes in a rat model of Parkinson's disease and cerebral infarction (12, 35). Therefore, appropriate preconditioning may enhance the effects of *trans*-differentiation on the restoration of degenerated tissues.

To our knowledge, this is the first report showing the therapeutic potential of MSCs for prion diseases. We showed that hMSCs home to the lesions, produce trophic factors, and differentiate into neuronal and glial lineage cells in response to the microenvironment in the lesions. As we are already aware, not only inhibition of prion propagation but also regeneration of damaged nervous tissues is required for recovery from prion diseases. Thus, a combination of genes possessing antiprion effects with MSCs, which can deliver therapeutic genes and have potential for neuroprotection and the regeneration of damaged tissues, may provide an effective treatment for prion diseases.

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