

In Vivo Tracking of Human Mesenchymal Stem Cells in Experimental Stroke

Daehong Kim,^{*1} Byoung-gi Chun,^{†1} Yeon-Kyung Kim,^{‡‡} Yong Hyun Lee,^{‡‡}
Cheong-Soo Park,^{*} Iksoo Jeon,[†] Chaejoon Cheong,^{*} Tae-Sun Hwang,[†] Hyungmin Chung,^{‡‡}
Byoung Joo Gwag,[§] Kwan Soo Hong,^{*} and Jihwan Song[†]

^{*}MRI Team, Korea Basic Science Institute, Ochang, Korea

[†]CHA Stem Cell Institute, Pochon CHA University College of Medicine, Seoul, Korea

^{‡‡}Stem Cell Therapy Institute, CHA Biotech Co., Ltd., Seoul, Korea

[§]Department of Pharmacology, Ajou University School of Medicine, Suwon, Gyeonggi-do, Korea

To understand the fates of human mesenchymal stem cells (hMSCs) following transplantation into a rodent model of middle cerebral artery occlusion (MCAo), magnetic resonance imaging (MRI) techniques were employed. hMSCs were labeled with ferumoxides (Feridex®)–protamine sulfate complexes, which were visualized and examined by MRI up to 10 weeks following transplantation. Migration of the transplanted cells to the infarcted area was further confirmed by histological methods. We found that the hMSCs transplanted in MCAo models possess the capacity to migrate to the infarcted area extensively in both ipsilateral and contralateral injections, exhibiting a pathotropism. We also analyzed the detailed migration patterns of transplanted hMSCs. We speculate that the extensive migratory ability of hMSCs may represent a therapeutic potential for developing efficient cell transplantation strategies in stroke.

Key words: Migration; Human mesenchymal stem cells (hMSCs); Middle cerebral artery occlusion (MCAo); Magnetic resonance imaging (MRI)

INTRODUCTION

The utilization of magnetic resonance imaging (MRI) approaches to noninvasively monitor cells that are labeled with MR contrast agents has been one of the major research directions in the past several years (1–3,7,10, 11). In particular, the ability to image animals repeatedly using MRI offers an invaluable opportunity to longitudinally follow implanted cells in vivo, allowing a direct assessment of cell migration. In this study, we used a 4.7 T animal MRI to follow up the fates of human mesenchymal stem cells (hMSCs) labeled with ferumoxides (Feridex®)–protamine sulfate complexes following transplantation into a rodent model of middle cerebral artery occlusion (MCAo) up to 10 weeks (3,16). Interestingly, we found that transplanted hMSCs were migrated to the lesioned sites extensively. In this report, we also described the detailed migration patterns of transplanted hMSCs.

MATERIALS AND METHODS

MCAo Animal Models and Preparation of hMSCs for Transplantation

MCAo was induced according to the method of Longa using Sprague-Dawley male rats, weighing 250–300 g (16). hMSCs (a gift from Drs. Sung-Soo Kim and Haeyoung Suh-Kim at Ajou University, Korea) were routinely maintained as previously described (12). For cell labeling, ferumoxide and protamine sulfate were initially prepared at a concentration of 2 µg/ml of DMEM each without serum, which were mixed for 30 min at room temperature and were subsequently added at an equal volume to the culture medium containing hMSCs. Cells were cultured with the mixture for 12–16 h at 37°C. At 7 days post-MCAo, a total of 100,000 hMSCs (50,000 cells/µl) were transplanted stereotactically into either the ipsilateral side (AP: +1.0 mm, ML: –2.0 mm, DV: –4.0 mm, from bregma) or the contralateral side

Received February 2, 2007; final acceptance July 16, 2007.

¹These two authors contributed to this work equally.

Address correspondence to Jihwan Song, D.Phil., CHA Stem Cell Institute, Pochon CHA University College of Medicine, 4th Floor Vision Bldg., 606-16 Yeoksam 1-dong, Kangnam-gu, Seoul 135-081, Republic of Korea. Tel: +82 2 3468 3393; Fax: +82 2 3468 3264; E-mail: jsong@cha.ac.kr or Kwan Soo Hong, Ph.D., MR Micro-Imaging Team, Korea Basic Science Institute, 804-1 Yangcheon-ri, Ochang-myun, Cheongwon-gun, Chungcheongbuk-do 363-883, Republic of Korea. Tel: +82 43 240 5100; Fax: +82 43 240 5069; E-mail: kshong@kbsi.re.kr

(AP: +0.5 mm, ML: +3.0 mm, DV: -4.0mm, from bregma) of the infarct region. In the control animals, in which 2 μ l of cell suspension medium (i.e., DMEM) was injected, stereotaxic coordinates were the same as those of contralateral injection. A total of 13 animals (ipsilateral: 6, contra-lateral: 5, control: 2) were used in this study. Based on the study of Li et al. (14), no immunosuppressive agents were given during the course of MRI examination following hMSC transplantation.

MRI Setting and Detection Methods

All experiments were performed using a 4.7 T Bio Spec (Bruker, Germany) with T1-, T2-, and T2*-weighted imaging techniques. To determine whether the infarcted area was visible by MRI, some rapid MRI examination was performed, which involved the acquisition of 20 axial slices of the brain with the T1-weighted spin echo sequence and T2-weighted rapid acquisition with relaxation enhancement (RARE) sequence. Acquisition parameters used were repetition time (T_R) = 600 ms (T1w)/5000 ms (T2w); echo time (T_E) = 14 ms (T1w)/ 90 ms (T2w); 256×192 matrix; field of view (FOV) = 4×3 cm; slice thickness = 1 mm; RARE factor = 8. T2*-weighted images were obtained using fast low angle shot (FLASH) sequence with the following acquisition parameters: T_R = 758 ms; T_E = 30 ms; geometry parameters used were the same as above. MRI examination was carried out regularly: initially at 2 days following transplantation, then at day 7, afterwards weekly up to 10 weeks. To understand the migration patterns in more detail, three-dimensional (3D) T2*-weighted imaging techniques were also applied at 10 weeks, in which acquisition parameters used were T_R = 560 ms; T_E = 20 ms; $256 \times 96 \times 32$ matrix; FOV = $4 \times 3 \times 1$ cm.

Histological Analyses

At 10 weeks following transplantation, histological examinations were carried out to analyze the transplanted cells. Prussian blue staining was used to detect the Feridex®-labeled hMSCs (3). Human-specific nuclei antibody (Chemicon, 1:500) was further used to confirm that the Prussian blue-positive cells were originated from the transplanted human cells. Additional immunohistochemical staining was carried out using the following antibodies: human-specific nestin (Chemicon, 1:200), type III β -tubulin (Chemicon, 1:500), MAP2 (Chemicon, 1:200), tyrosine hydroxylase (Pel-Freeze, 1:1,000), and GFAP (Santa Cruz, 1:1,000).

RESULTS

Detection and Migration of Feridex®-Labeled hMSCs Using MRI

The amounts of irons incorporated in each cell were shown to be 2.6 pg Fe/cell, according to the ICP/MS

(Inductively Coupled Plasma Mass Spectrometer; Thermo Elemental, USA). To study the migratory effects of MSC, we transplanted Feridex®-labeled hMSC at different locations in MCAo animal models, and followed their migration patterns up to 10 weeks using MRI. Figure 1 shows some representative serial T2*-weighted MR images taken from 2 days and 1, 2, 4, 6, 8, and 10 weeks following transplantation, respectively (equivalent T1- and T2-weighted images were also obtained but not shown here). Interestingly, we found that there is a strong tendency of transplanted hMSCs to migrate towards the infarcted area, regardless of injection sites [i.e., ipsilateral (Fig. 1A) vs. contralateral (Fig. 1B)]. However, migration of the transplanted hMSCs was hardly detectable in the control (Fig. 1C). When examined by MRI, in both ipsilateral and contralateral injections, the labeled cells started showing indications of migration as early as 1 or 2 weeks following transplantation in our case, after which the MRI signals were gradually intensified and extended towards the infarcted area. At 10 weeks, the majority of labeled cells were detected not only at the boundary but also in the core of infarcted area. In the latter case, the labeled cells were found in various regions in the basal ganglia (Fig. 1A, B). Interestingly, in the case of contralateral injection, we observed that the labeled cells were mainly detected close to the infarcted area and no particular signal was detected in other places. The migratory nature of transplanted hMSCs in MCAo animal models was shown to be consistent in all animals examined throughout this study. As for the remaining signals found in the original injection site and its vicinity, we performed immunohistochemical staining using human-specific nuclei antibody and found that they do not correspond to the transplanted cells (data not shown). We speculate that these image artifacts may result from the residual Feridex® particles at the injection site.

Migration Patterns of Feridex®-Labeled hMSCs

In addition to MRI analysis, we carried out histological examinations at 10 weeks in order to understand the migration patterns of transplanted hMSCs to the infarcted area. Figure 2A shows one original MR image and its corresponding histological analyses when Feridex®-labeled hMSCs were transplanted into the contralateral side of MCAo animal models. As shown in Figure 1B, strong MRI signals were detected at the boundary of infarct cavity. Prussian blue staining, which can detect Feridex®-labeled cells by recognizing iron oxides specifically, further provided the evidence that the transplanted hMSCs were migrated to the opposite hemisphere and were detected clearly in various sites of the infarcted area (Fig. 2A-a). We also carried out immunohistochemical staining using an antibody against

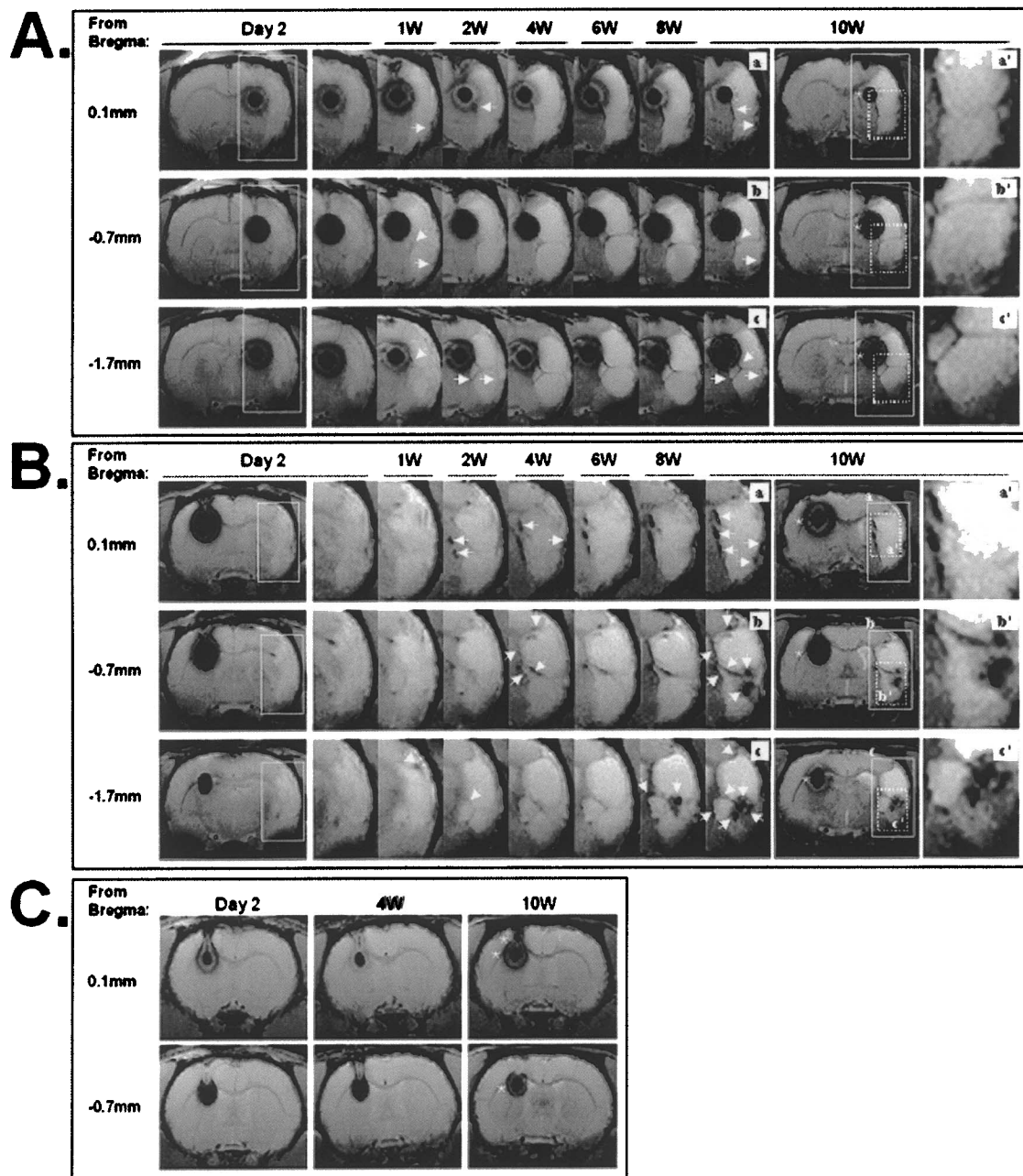


Figure 1. Detection and migration of Feridex®-labeled hMSCs using MRI when they were transplanted into the ipsilateral (A) or contralateral (B) side of MCAo animal models, or into one hemisphere of the control animal (C). Some representative T2*-weighted MR images at the positions (0.1 ~ -1.7 mm from bregma), in which labeled stem cells were highly detected, were taken at 2 days and 1, 2, 4, 6, 8, and 10 weeks following transplantation. Dark circles indicate the transplanted cells, whereas arrows indicate the migrating cells. To avoid complexity, only the initial and final stages of detection were highlighted. At 10 weeks, both outer rectangles (a, b, c) and inner rectangles (a', b', c') were created in order to highlight the labeled cells inside the infarcted area at higher magnifications. Asterisks indicate image artifacts caused by residual Feridex® particles. Note that extensive cell migration was detected in (A) and (B), but not in (C).

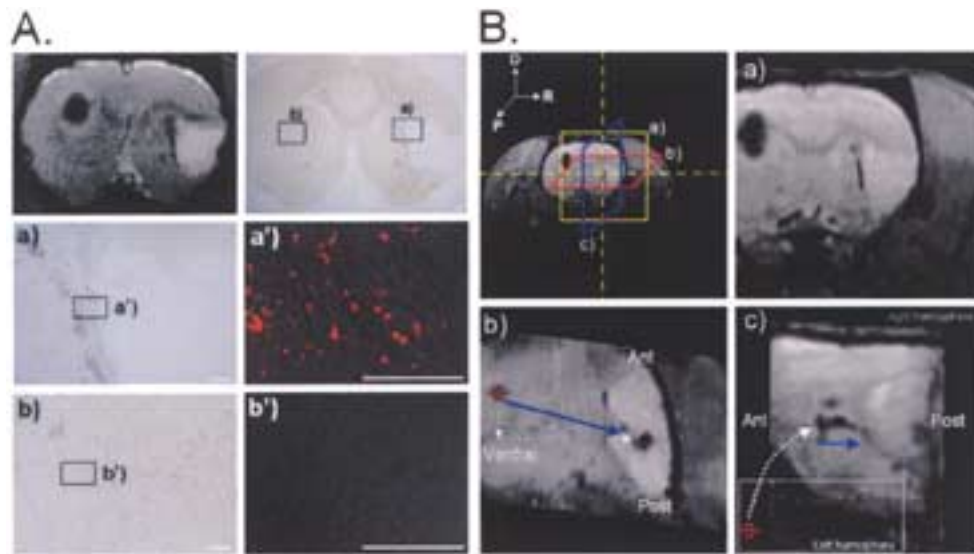


Figure 2. Migration patterns of Feridex®-labeled hMSCs when transplanted into the contralateral side of MCAo animal models. (A) Original MR image and its corresponding histological analyses. Arrowheads indicate representative MRI signals. Feridex®-labeled hMSCs were clearly detectable in the infarcted area, visualized by Prussian Blue staining (a). Immunohistochemical staining using human-specific nuclei shows that they were originated from the transplanted human cells (a'). No particular signals for either Prussian blue (b) or human-specific nuclei (b') were detected in the intact brain hemisphere. Scale bar: 100 μ m. (B) 3D T2*-weighted image showing the migratory path (indicated as asterisks). (a) Coronal image, showing the original transplantation site (left hemisphere) and the migrated stem cells (right hemisphere). (b) Horizontal image, showing the ventro-posterior migration. (c) Sagittal image of the right hemisphere; anterior-to-posterior migration of the cells is indicated as an arrow. Abbreviations: D, dorsal; R, right; P, posterior; cc, corpus callosum; Ant, anterior; Post, posterior.

human-specific nuclei, and found that the Prussian blue-positive cells were originated from the transplanted human cells (Fig. 2A-a'). In contrast, no particular signals for either Prussian blue (Fig. 2A-b) or human-specific nuclei (Fig. 2A-b') were detected in the intact brain hemisphere when examined at 10 weeks after transplantation. We then attempted to characterize the cell types using various neuronal and glial markers, including nestin, type III β -tubulin, MAP2, tyrosine hydroxylase, and GFAP, but no double-labeled cells were detected (data not shown). Similar results were observed when Feridex®-labeled hMSCs were transplanted into the ipsilateral side of infarcted area (data not shown). To determine the detailed migration patterns, we performed 3D T2*-weighted image analyses (Fig. 2B). It appears that the migration patterns of transplanted cells from the injection site to the infarcted area were extraordinarily dynamic. According to our observations, it appears that the contralaterally transplanted hMSCs initially crossed the border of each hemisphere, presumably through corpus callosum (Fig. 2B-a), and eventually reached the infarcted area ventro-posteriorly (Fig. 2B-a, b). Interestingly, the labeled cells were detected not only at the periphery but also in the core of the infarcted area. Moreover, it is noteworthy that a group of labeled cells

migrated extensively to the posterior direction (Fig. 2B-c).

DISCUSSION

Over the past few years, there has been great interest in the potential of stem cells for the treatment of stroke, and recent clinical reports have indeed shown some functional improvements following stem cell transplantation in stroke patients (5). However, there has been no direct evidence showing a proof of principle, in which the transplanted stem cells directly gave rise to a functional recovery. In this study, we showed that the fates of transplanted human mesenchymal stem cells (hMSCs) can be efficiently monitored by using a 4.7 T animal MRI. We found that Feridex®-labeled hMSCs can survive at least up to 10 weeks following transplantation into a rodent model of MCAo, of which MRI results were further confirmed by histological methods. It is interesting to note that the transplanted MSCs were shown to survive in the absence of immunosuppressive agents, in agreement with the study of Li et al. (14), in which hMSCs were shown to induce the proliferation of rat lymphocytes without the induction of rat cytotoxic T lymphocytes.

According to our observations, it appears that there

is a strong tendency of transplanted hMSCs to migrate towards the infarcted area, regardless of injection sites (Fig. 1A, B). In particular, contralaterally transplanted hMSCs exhibited extremely dynamic migration patterns, as described above (Figs. 1B, 2B). In some cases, it looks as if slightly more cells were migrated when transplanted contralaterally. However, it appears that degrees of cell migration detected by MRI were variable, presumably due to the differences in ischemic condition in each animal. Interestingly, the labeled cells were detected not only at the boundary but also in the core of infarct cavity, implying the possibility of infiltration of the transplanted cells, which will be necessary for tissue remodeling and neural regeneration in the damaged brain (9,13). Migration of transplanted hMSCs has been reported previously (4,21). It is also known that neural stem cells exhibit pathotropism in either brain ischemic or tumor animal models (6,18). More recently, while this work was being prepared, a similar study using MRI was published (19) in which non-iron-labeled immortalized human MSCs migrated across the midline to the contralateral corpus callosum and hippocampus. Although the experimental details are quite different, we believe that the recent study also supports our observations.

Therapeutic effects of MSCs in stroke animal models have been reported, but the mechanisms underlying functional recovery are largely unknown (14,21). Moreover, neurogenic potential of MSCs in vitro and in vivo is still controversial (8,15,17,20). In the present study, transplanted hMSCs, despite their extensive migratory capacity, were not able to differentiate into neural tissues efficiently, judged by lack of neuronal marker expression (data not shown). Therefore, it will be difficult to speculate that the long-distance migrated hMSC gave rise to a neuronal regeneration. On the other hand, according to our preliminary analysis from MR spectroscopy experiments, the ratio of lactate to *N*-acetyl-aspartate was shown to decrease gradually following hMSCs transplantation (data not shown), which is currently under verification. To better achieve therapeutic purposes, other cell sources, including neural stem cells or embryonic stem cells, can be also taken into consideration (9,13).

Taken together, the present MRI study provides useful information on the fates and migration patterns of hMSCs when they were transplanted into stroke animal models. We speculate that the extensive migratory nature of stem cells and its utilizations will provide an important tool to develop novel stroke therapeutics, including stem cell therapy (6,18).

ACKNOWLEDGMENTS: This work was supported by grants from Korea Food & Drug Administration, Republic of Korea (07102-KFDA-428) and Korea Research Foundation, Repub-

lic of Korea (MOEHRD) (KRF-2005-041-E00334) to J.S., a grant from the Center for Biological Modulators of the 21st Century Frontier R&D Program, Republic of Korea (CBM2-B611-001-2-1-0) to K.S.H., and a grant from the Brain Research Center funded by the Korean Ministry of Science and Technology (R11-1998-052-07007-0) to B.J.G. The authors also wish to express their special thanks to anonymous reviewers, who helped us to improve this work significantly.

REFERENCES

1. Arbab, A. S.; Bashaw, L. A.; Miller, B. R.; Jordan, E. K.; Bulte, J. W.; Frank, J. A. Intracytoplasmic tagging of cells with ferumoxides and transfection agent for cellular magnetic resonance imaging after cell transplantation: Methods and techniques. *Transplantation* 76:1123–1130; 2003.
2. Arbab, A. S.; Yocum, G. T.; Kalish, H.; Jordan, E. K.; Anderson, S. A.; Khakoo, A. Y.; Read, E. J.; Frank, J. A. Efficient magnetic cell labeling with protamine sulfate complexed to ferumoxides for cellular MRI. *Blood* 104: 3410–3412; 2004.
3. Arbab, A. S.; Yocum, G. T.; Rad, A. M.; Khakoo, A. Y.; Fellowes, V.; Read, E. J.; Frank, J. A. Labeling of cells with ferumoxides-protamine sulfate complexes does not inhibit function or differentiation capacity of hematopoietic or mesenchymal stem cells. *NMR Biomed.* 18:553–559; 2005.
4. Azizi, S. A.; Stokes, D.; Augelli, B. J.; DiGirolamo, C.; Prockop, D. J. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats—similarities to astrocyte grafts. *Proc. Natl. Acad. Sci. USA* 95:3908–3913; 1998.
5. Bang, O. Y.; Lee, J. S.; Lee, P. H.; Lee, G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann. Neurol.* 57:874–882; 2005.
6. Bhakta, S.; Hong, P.; Koc, O. The surface adhesion molecule CXCR4 stimulates mesenchymal stem cell migration to stromal cell-derived factor-1 in vitro but does not decrease apoptosis under serum deprivation. *Cardiovasc. Revasc. Med.* 7:19–24; 2006.
7. Bulte, J. W.; Douglas, T.; Witwer, B.; Zhang, S. C.; Strable, E.; Lewis, B. K.; Zywicke, H.; Miller, B.; van Gelderen, P.; Moskowitz, B. M.; Duncan, I. D.; Frank, J. A. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. *Nat. Biotechnol.* 19: 1141–1147; 2001.
8. Deng, J.; Petersen, B.; Steindler, D. Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. *Stem Cells* 24:1054–1064; 2006.
9. Goldman, S. Stem and progenitor cell-based therapy of the human central nervous system. *Nat. Biotechnol.* 23: 862–871; 2005.
10. Hoehn, M.; Kustermann, E.; Blunk, J.; Wiedermann, D.; Trapp, T.; Wecker, S.; Focking, M.; Arnold, H.; Heschler, J.; Fleischmann, B. K.; Schwindt, W.; Buhrl, C. Monitoring of implanted stem cell migration in vivo: A highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat. *Proc. Natl. Acad. Sci. USA* 99:16267–16272; 2002.
11. Kim, D.; Hong, K. S.; Song, J. The present status of cell tracking methods in animal models using magnetic resonance imaging technology. *Mol. Cells* 23:132–137; 2007.
12. Kim, S. S.; Choi, J. M.; Kim, J. W.; Ham, D. S.; Ghil, S. H.; Kim, M. K.; Kim-Kwon, Y.; Hong, S. Y.; Ahn,

- S. C.; Kim, S. U.; Lee, Y. D.; Suh-Kim, H. cAMP induced neuronal differentiation of mesenchymal stem cells via activation of extracellular signal-regulated kinase/MAPK. *Neuroreport* 16:1357–1361; 2005.
13. Lindvall, O.; Kokaia, Z. Stem cells for the treatment of neurological disorders. *Nature* 441:1094–1096; 2006.
 14. Li, Y.; Chen, J.; Chen, X. G.; Wang, L.; Gautam, S. C.; Xu, Y. X.; Katakowski, M.; Zhang, L. J.; Lu, M.; Janakiraman, N.; Chopp, M. Human marrow stromal cell therapy for stroke in rat: Neurotrophins and functional recovery. *Neurology* 59:514–523; 2002.
 15. Li, Y.; Chen, J.; Chopp, M. Adult bone marrow transplantation after stroke in adult rats. *Cell Transplant.* 10:31–40; 2001.
 16. Longa, E. Z.; Weinstein, P. R.; Carlson, S.; Cummins, R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 20:84–91; 1989.
 17. Lu, P.; Blesch, A.; Tuszynski, M. Induction of bone marrow stromal cells to neurons: Differentiation, trans-differentiation, or artifact? *J. Neurosci. Res.* 77:174–191; 2004.
 18. Muller, F. J.; Snyder, E. Y.; Loring, J. F. Gene therapy: Can neural stem cell deliver? *Nat. Rev. Neurosci.* 7:75–84; 2006.
 19. Shyu, W. C.; Chen, C. P.; Lin, S. Z.; Lee, Y. J.; Li, H. Efficient tracking of non-iron-labeled mesenchymal stem cells with serial MRI in chronic stroke rats. *Stroke* 38:367–374; 2006.
 20. Wehner, T.; Bontert, M.; Eyupoglu, I.; Prass, K.; Prinz, M.; Klett, F. F.; Heinze, M.; Bechmann, I.; Nitsch, R.; Kirchhoff, F.; Kettenmann, H.; Dirnagl, U.; Priller, J. 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.* 23:5004–5011; 2003.
 21. Zhao, L. R.; Duan, W. M.; Reyes, M.; Keene, C. D.; Verfaillie, C. M.; Low, W. C. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp. Neurol.* 174:11–20; 2002.