

# *In vivo* Tracking of Human Neural Stem Cells Following Transplantation into a Rodent Model of Ischemic Stroke

Da-Jeong Chang<sup>1\*</sup>, Hyeyoung Moon<sup>2\*</sup>, Yong Hyun Lee<sup>1</sup>, Nayeon Lee<sup>1</sup>,  
Hong J. Lee<sup>3</sup>, Iksoo Jeon<sup>1</sup>, Hyunseung Lee<sup>2</sup>, Tae-Sun Hwang<sup>1</sup>, Seung-Hun Oh<sup>1</sup>,  
Dong Ah Shin<sup>4</sup>, Seung U. Kim<sup>3</sup>, Kwan Soo Hong<sup>2</sup>, Jihwan Song<sup>1</sup>

<sup>1</sup>CHA Stem Cell Institute, Department of Biomedical Science, CHA University, Seoul,

<sup>2</sup>Division of Magnetic Resonance, Korea Basic Science Institute, Ochang,

<sup>3</sup>Medical Research Institute, Chung-Ang University College of Medicine,

<sup>4</sup>Department of Neurosurgery, Yonsei University College of Medicine, Seoul, Korea

**Background and Objectives:** Ischemic stroke caused by middle cerebral artery occlusion (MCAo) is the major type of stroke, but there are currently very limited options for cure. It has been shown that neural stem cells (NSCs) or neural precursor cells (NPCs) can survive and improve neurological deficits when they are engrafted in animal models of various neurological diseases. However, how the transplanted NSCs or NPCs are act *in vivo* in the injured or diseased brain is largely unknown. In this study, we utilized magnetic resonance imaging (MRI) techniques in order to understand the fates of human NSCs (HB1.F3) following transplantation into a rodent model of MCAo.

**Methods and Results:** HB1.F3 human NSCs were pre-labeled with ferumoxides (Feridex<sup>®</sup>)-protamine sulfate complexes, which were visualized and examined by MRI up to 9 weeks after transplantation. Migration of the transplanted cells to the infarct area was further confirmed by histological methods.

**Conclusions:** Based on these observations, we speculate that the transplanted NSCs have the extensive migratory ability to the injured site, which will in turn contribute to functional recovery in stroke.

**Keywords:** Human neural stem cells (hNSCs), Ischemic stroke, Middle cerebral artery occlusion (MCAo), Magnetic resonance imaging (MRI), Feridex, *In vivo* tracking

## Introduction

Stroke is one of the leading causes of adult death and disability worldwide. Ischemic stroke is caused by blockade of the blood supply to part of the brain. Despite numerous research efforts, there are still currently very limited ther-

apeutic options for the stroke-damaged patients. It has been shown that neural stem cells (NSCs) or neural precursor cells (NPCs) engrafted in animal models of various neurological diseases can survive and improve neurological deficits (1, 2). We have previously established a clonal human neural stem cell line, HB1.F3 (briefly called F3 thereafter), which was immortalized by a retroviral vector encoding *v-myc* oncogene (3, 4). The F3 cells showed a multipotent capacity to differentiate into neurons and glial cells (3, 4) and were able to ameliorate the neurological deficits in animal models of stroke (5-8). However, compared with these differentiation and therapeutic potentials, how the transplanted NSCs act in order to exert such effects *in vivo* is largely unknown. In this regard, it is worthwhile to utilize magnetic resonance imaging (MRI) approaches to

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Correspondence to **Jihwan Song**

CHA Stem Cell Institute, Department of Biomedical Science, CHA University, 606-16 Yeoksam 1-dong, Gangnam-gu, Seoul 135-081, Korea

Tel: +82-2-3468-3393, Fax: +82-2-538-4102

E-mail: jsong@cha.ac.kr

\*These authors contributed equally to this work.

non-invasively monitor cells that are labeled with MR contrast agent, which has been one of the major research directions in the past decade (9-13). In particular, the ability to image animals repeatedly using MRI can serve as useful tools to monitor the presence, migration and distribution patterns of transplanted cells *in vivo*. In this study, we employed a 4.7 T animal MRI to follow up the fates of F3 human NSC labeled with ferumoxides (Feridex<sup>®</sup>)-protamine sulfate complexes following transplantation into a rodent model of middle cerebral artery occlusion (MCAo) up to 9 weeks. Interestingly, we found that the majority of transplanted cells were migrated to the infarct area, showing a patho-tropism. In this study, we also described the detailed migration patterns of transplanted hNSCs.

## Materials and Methods

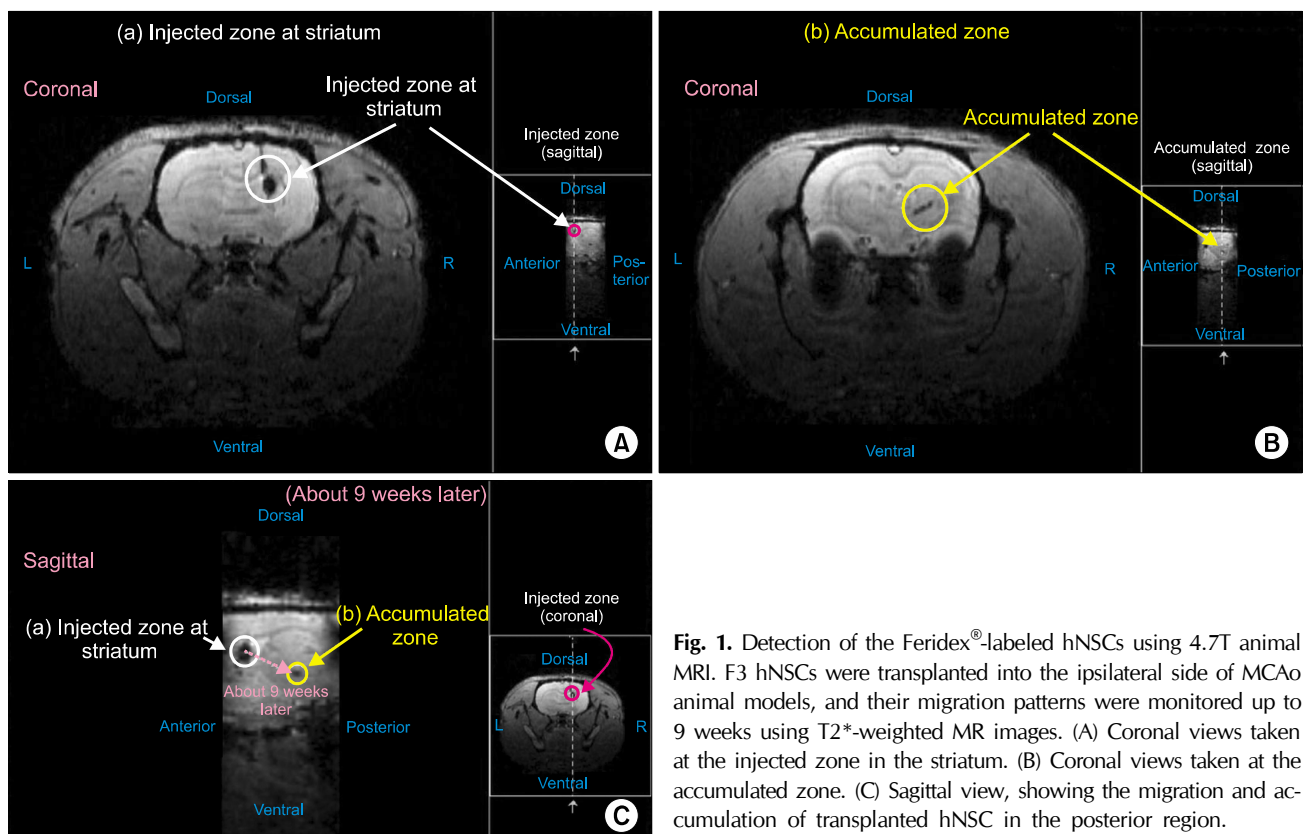
### MCAo animal models

All of the experimental animals were manipulated in accordance with the CHA University IACUC (Institutional Animal Care and Use Committee; IACUC090012). Adult male Sprague-Dawley rats (n=8) weighing 250~300 g (Orient, Seoul, Korea) were used in this experiment. After anesthesia with 1% ketamine (30 mg/kg, i.p) and xylazine

hydrochloride (4 mg/kg, i.p), body temperature of rats were maintained at  $37\pm 1^{\circ}\text{C}$  by a rectal probe and heating pad. According to the method of Longa, animals were subjected to temporary middle cerebral artery occlusion (MCAo), which was induced by the retrograde insertion of a 3-0 poly-L-lysine-coated nylon suture via the external carotid artery into the internal carotid artery and MCA. Reperfusion carried out by removing the suture 60 min later (14).

### Preparation of F3 hNSCs for transplantation

For cell labeling, ferumoxide and protamine sulfate were initially prepared at a concentration of  $2\ \mu\text{g}/\text{ml}$  of DMEM each without serum, which were subsequently mixed for 30 min at room temperature and were added with an equal volume to the culture medium containing F3 hNSCs (13). Cells were cultured with the mixture for 12~16 hr at  $37^{\circ}\text{C}$ . At 7 days after the induction of MCAo, cells were stereotaxically transplanted into the striatum (n=4) using the following conditions:  $1\times 10^5$  cells in  $2\ \mu\text{l}$  (AP: +1.0 mm, ML: -1.5 mm, DV: -4.0 mm). As controls (n=4), a vehicle containing medium was transplanted into the same coordinates. Transplanted animals were received cyclosporine A (Sigma, 10 mg/kg, i.p) 24 h before transplantation and daily up to 9 weeks.



**Fig. 1.** Detection of the Feridex<sup>®</sup>-labeled hNSCs using 4.7T animal MRI. F3 hNSCs were transplanted into the ipsilateral side of MCAo animal models, and their migration patterns were monitored up to 9 weeks using T2\*-weighted MR images. (A) Coronal views taken at the injected zone in the striatum. (B) Coronal views taken at the accumulated zone. (C) Sagittal view, showing the migration and accumulation of transplanted hNSC in the posterior region.

### MRI detection of transplanted cells

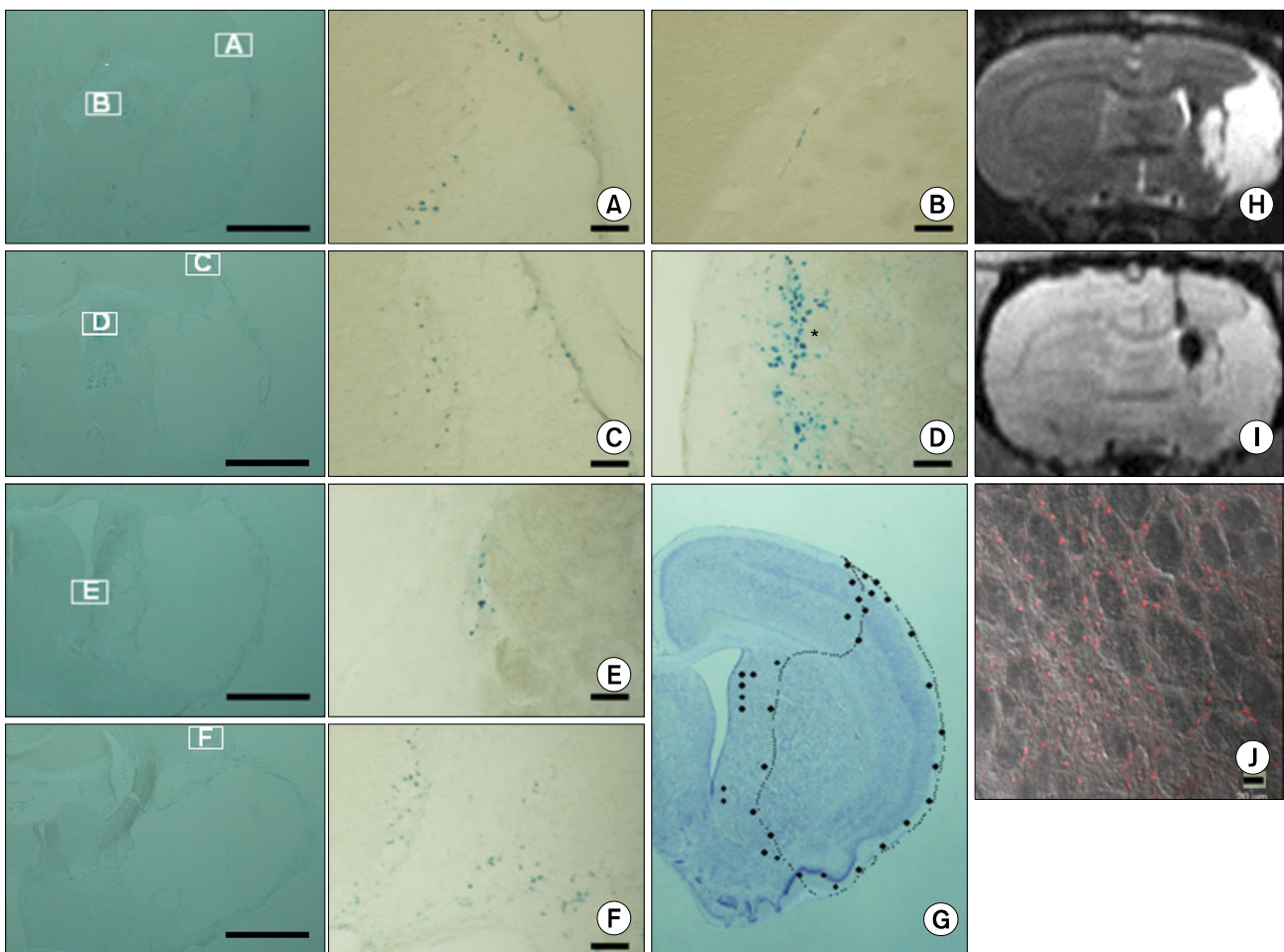
To detect the Feridex<sup>®</sup>-labeled F3 hNSCs, we employed a 4.7T Bio Spec (Bruker, Germany) for animal MRI analysis using T2- and T2\*-weighted imaging techniques. The MRI setting and detection methods were the same as described previously (13). To detect the transplanted stem cells, Prussian blue staining was carried out at 9 weeks following transplantation as described (13), followed by immunohistochemical staining using an antibody against human specific-nuclei (Chemicon, 1 : 500).

### Results

#### Detection and migration of the Feridex<sup>®</sup>-labeled hNSCs using MRI

To investigate the migratory effects of NSC, we trans-

planted Feridex<sup>®</sup>-labeled hNSC into the ipsilateral side of MCAo animal models, and followed their migration patterns up to 9 weeks using MRI. Fig. 1A and 1B show the representative coronal views of T2\*-weighted MR images taken from 9 weeks following transplantation. Interestingly, cells transplanted into the striatum (Fig. 1A) were also detected and accumulated in the posterior region (Fig. 1B). Fig. 1C is the sagittal view of T2\*-weighted images, showing the migration and accumulation of transplanted hNSC in the posterior region. Taken together, these coronal and sagittal views of T2\*-weighted MR images clearly show that the transplanted hNSCs migrated to the infarct region in anterior-posterior and dorsal-ventral directions.



**Fig. 2.** Histological analyses of Feridex<sup>®</sup>-labeled hNSCs. (A~F) Prussian blue staining showing the presence and distribution of Feridex<sup>®</sup>-labeled hNSCs. Left panel, lower magnification; A~F, high magnification. (G) Distribution patterns of Feridex<sup>®</sup>-labeled hNSC, showing the great majority of transplanted cells were localized at the periphery of infarct region. (H, I) Coronal views of T2 and T2\*-weighted images, providing the gross morphology of infarct area and the location of transplanted cells, respectively. (J) Immunohistochemical staining showing the presence of human cells. Scale bars: left panel, 1 mm; A~F, 100 μm, J, 20 μm.

### Migration patterns of Feridex<sup>®</sup>-labeled hNSCs

In addition to MRI analysis, we carried out histological examinations at 9 weeks in order to understand the migration patterns of transplanted hNSCs to the infarcted area. Fig. 2 shows the results of Prussian blue staining (left panel, lower magnification; Fig. 2A-F, high magnification). Fig. 2G shows the distribution patterns of Feridex<sup>®</sup>-labeled hNSC, indicating the great majority of transplanted cells were localized at the periphery of infarct region. Fig. 2H and 2I show coronal view of T2 and T2\*-weighted images, providing the gross morphology of infarct area and the location of transplanted cells, respectively. Fig. 2J shows that the Feridex<sup>®</sup>-containing cells (i.e., MRI-positive cells) were indeed derived from human NSCs, judged by the positive staining for an antibody against human-specific nuclei. Taken together, these results clearly indicate the transplanted hNSC migrated to the periphery of infarct region in a ventro-posterior direction.

### Discussion

Over the past several years, there has been the great interest in the potential of stem cells for treating stroke, and studies from animal experiments and clinical trials using various cell types in recent years strongly suggest that stem cell therapy would be one of the legitimate options for the treatment of stroke (15-17). In this study, we showed that the fates of transplanted F3 hNSCs in a rodent model of ischemic stroke can be efficiently monitored by using an animal MRI. We found that the Feridex<sup>®</sup>-labeled hNSCs can survive up to 9 weeks after transplantation and were migrated to the infarct region, of which results were further confirmed by histological methods using Prussian blue staining, as well as immunohistochemical staining using a human-specific antibody. According to our observations, there is a strong tendency of transplanted hNSCs to migrate to the infarct area, showing a strong patho-tropism, which has been also observed in animal models of various other diseases, including brain tumors. Several studies have reported that neural stem cells or neural precursor cells express the chemokine receptor, CXCR4, which has the high affinity to SDF-1 (stromal cell-derived factor-1) that is strongly released in the ischemic boundary zone of the brain (18, 19).

Previous studies using F3 hNSCs indicate that they can differentiate into neurons and astrocytes and possess electrophysiological characteristics of mature neurons (20). It is also known that F3 hNSCs can secrete some neurotrophic factors (4). Therefore, it is quite likely that all of these properties will contribute to the neural protection

and/or neuronal regeneration of the ischemic tissues, as well as to the amelioration of behavioral deficits in stroke animal models. Therefore, in order to develop efficient stroke cell therapy, it will be essential to determine the optimal cell types and transplantation conditions.

Taken together, the present MRI study provides useful information on the migratory routes of hNSCs following transplantation into a rodent model of ischemic stroke. Therefore, in combination with behavioral tests, *in vivo* tracking method using MRI will provide useful tools to closely monitor the action mode of transplanted stem cells and the extent of functional recovery following stem cell transplantation non-invasively.

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### Potential conflict of interest

The authors have no conflicting financial interest.

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