We aimed to identify a suitable method for long-term monitoring of the migration and proliferation of mesenchymal stromal cells in stroke models of rats using ferritin transgene expression by magnetic resonance imaging (MRI). Bone marrow mesenchymal stromal cells (BMSCs) were transduced with a lentivirus containing a shuttle plasmid (pCDH-CMV-MCS-EF1-copGFP) carrying the ferritin heavy chain 1 (Fth1) gene. Ferritin expression in stromal cells was evaluated with western blotting and immuno-fluorescent staining. The iron uptake of Fth1-BMSCs was measured with Prussian blue staining. Following surgical introduction of middle cerebral artery occlusion, Fth1-BMSCs and superparamagnetic iron oxide- (SPIO-) labeled BMSCs were injected through the internal jugular vein. The imaging and signal intensities were monitored by diffusion-weighted imaging (DWI), T2-weighted imaging (T2WI), and susceptibility-weighted imaging (SWI) \textit{in vitro} and \textit{in vivo}. Pathology was performed for comparison. We observed that the MRI signal intensity of SPIO-BMSCs gradually reduced over time. Fth1-BMSCs showed the same signal intensity between 10 and 60 days. SWI was more sensitive than T2WI and suitable as the optimal MRI-tracking sequence.

1. Introduction

Magnetic resonance imaging (MRI) is an important tool for cellular imaging as it can be used for clinical diagnostics. Current therapies for disorders due to ischemia reperfusion are unable to repair damaged or lost neural cells because neural cells are nonproliferative cells and limited in number. Bone marrow mesenchymal stromal cells (BMSCs) have been used for several regenerative methods in animal models or patients [1]. Nevertheless, the fate of transplanted BMSCs in live animals is still poorly understood [2–4]. Thus, a noninvasive, real-time, sensitive, and clinically applicable method for tracking transplanted BMSCs and monitoring their behavior in live animals would be useful. Superparamagnetic iron oxide (SPIO) has been used in several studies for tracking BMSCs \textit{in vitro} and \textit{in vivo} [1, 5, 6]. However, the hypointense MRI signal generated by these particles is not sustainable over a long time because the iron oxide nanoparticles are diluted with each cell division. Moreover, SPIO nanoparticles can be targeted and cleared by macrophages [7, 8]. Consequently, BMSCs labeled with SPIO may not be a suitable method for long-term tracking of

Research Article

MRI Tracking of SPIO- and Fth1-Labeled Bone Marrow Mesenchymal Stromal Cell Transplantation for Treatment of Stroke

Xiaolei Huang,1 Yang Xue,2 Jinliang Wu,2 Qing Zhan2,4 and Jiangmin Zhao3,5

1Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai 201900, China
2Shanghai 6th People’s Hospital, Shanghai Jiao Tong University, Shanghai 200233, China
3Tianjin Medical University Hospital for Metabolic Diseases, Tianjin 300070, China
4Department of Neurology, Seventh People’s Hospital of Shanghai University of TCM, Shanghai 200137, China
5Department of Medical Imaging, Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200010, China

Correspondence should be addressed to Qing Zhan; zhanqing@tongji.edu.cn and Jiangmin Zhao; johnmzhao@sjtu.edu.cn

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1Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai 201900, China
2Shanghai 6th People’s Hospital, Shanghai Jiao Tong University, Shanghai 200233, China
3Tianjin Medical University Hospital for Metabolic Diseases, Tianjin 300070, China
4Department of Neurology, Seventh People’s Hospital of Shanghai University of TCM, Shanghai 200137, China
5Department of Medical Imaging, Shanghai 9th People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200010, China

Correspondence should be addressed to Qing Zhan; zhanqing@tongji.edu.cn and Jiangmin Zhao; johnmzhao@sjtu.edu.cn

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We aimed to identify a suitable method for long-term monitoring of the migration and proliferation of mesenchymal stromal cells in stroke models of rats using ferritin transgene expression by magnetic resonance imaging (MRI). Bone marrow mesenchymal stromal cells (BMSCs) were transduced with a lentivirus containing a shuttle plasmid (pCDH-CMV-MCS-EF1-copGFP) carrying the ferritin heavy chain 1 (Fth1) gene. Ferritin expression in stromal cells was evaluated with western blotting and immuno-fluorescent staining. The iron uptake of Fth1-BMSCs was measured with Prussian blue staining. Following surgical introduction of middle cerebral artery occlusion, Fth1-BMSCs and superparamagnetic iron oxide- (SPIO-) labeled BMSCs were injected through the internal jugular vein. The imaging and signal intensities were monitored by diffusion-weighted imaging (DWI), T2-weighted imaging (T2WI), and susceptibility-weighted imaging (SWI) \textit{in vitro} and \textit{in vivo}. Pathology was performed for comparison. We observed that the MRI signal intensity of SPIO-BMSCs gradually reduced over time. Fth1-BMSCs showed the same signal intensity between 10 and 60 days. SWI was more sensitive than T2WI and suitable as the optimal MRI-tracking sequence. SWI was more sensitive than T2WI and suitable as the optimal MRI-tracking sequence.
BMSC engraftment. Tracking reporter genes which express MRI-detectable proteins are a newly developing approach to monitor transplanted cells [9]. Ferritin, a ubiquitously expressed protein in vivo, can be visualized by MRI when overexpressed and can store iron in a nontoxic manner [7, 10–12]. In our study, we utilized a lentivirus carrying the ferritin heavy chain 1 (Fth1) gene [13], which encodes the ferritin protein to tag BMSCs, and then tracked the survival, migration, and proliferation of transplanted cells over a period of time.

2. Materials and Methods

2.1. Culture and Identification of BMSCs. All interventions and animal care procedures were performed in accordance with the Laboratory Animal Welfare Act, Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD), and Guidelines and Policies for Animal Surgery provided by our hospital (Shanghai jiaotong University, Shanghai, China) and were approved by the Institutional Animal Use and Care Committee.

BMSCs were isolated and cultured from the whole bone marrow of Sprague Dawley (SD) rats using the direct adherence method. The culture medium was replaced after the first 8 h and then changed every other day. We chose cells passaged for 3–5 generations for our experiments. To determine whether BMSCs expressed the surface marker, flow cytometric analysis of BMSCs was performed five independent times. In brief, the cells were incubated with PE-CD34, PE-CD44, and FITC-CD90 (AbD Serotec, Germany) antibodies for 30 min. Fluorescence-associated cell sorting was performed using a BD LSR II benchtop analyzer (BD Biosciences, America) for flow cytometry. To evaluate whether the BMSCs possessed differentiation capability, BMSCs were treated with [1] induction media (Cyagen, Guangzhou, China) to test the differentiation capability. The morphology change in BMSCs was analyzed.

2.2. Vector Design and Viral Production. Fth1 was amplified by PCR from the genomic DNA of rats to construct the overexpression lentiviral vector pCDH-CMV-MCS-EF1-copGFP-Fth1. The open reading frame (ORF) of Fth1 was designed to contain the restriction enzyme sites needed for cloning. For viral production, the transfer plasmid pCDH-CMV-MCS-EF1-copGFP-Fth1 (expressing the proteins gag, env, and FTH1) was transiently cotransfected with the packaging plasmid psPAX2 (Addgene 12259, expressing the protein VSV-G). The reaction program was set as 95°C/30s, 40 cycles of 95°C/5s, 95°C/15s, 60°C/15s, 70°C/10s, and then cooling at 4°C. We analyzed the relative gene expression data using the 2−ΔΔCT method.

2.3. BMSC Culture and Lentiviral Transduction. Three million of the supernatant of Fth1 lentivirus were mixed with an equal volume of the complete medium (DMEM/F-12 supplemented with 15% fetal bovine serum), which served as a lentiviral transduction compound medium. Before lentiviral transduction, 3rd or 4th passage BMSCs were grown to 80%–90% confluence, and then 3 × 10⁷ cells were cultured for 24 h with the compound medium. After that, the compound medium was replaced with the DMEM/F-12 medium. The transduction efficiency was assessed by the expression of EGFP after 3–4 d. The Fth1-BMSCs with EGFP expression efficiency over 70% were maintained and propagated for subsequent experiments.

2.4. Fluorescence Quantitative Real-Time PCR. Fluorescence quantitative real-time PCR (SYBR Premix Ex Taq. TaKaRa, JP) was used to assess the expression of the reporter gene Fth1. The reaction program was set as 95°C/30s, 40 cycles of 95°C/5s, 95°C/15s, 60°C/15s, 70°C/10s, and then cooling at 4°C. We analyzed the relative gene expression data using the 2−ΔΔCT method.

2.5. Western Blot. In order to assess the protein levels produced by translation of the Fth1 mRNA, BMSCs were transfected with the Fth1 lentivirus, and after 7 d, they were lysed in the prechilled lysis buffer (without bromophenol blue) with 50 μl PMSF (10 mg/ml) and homogenized with a sonicator. Proteins were extracted, quantified, and run on a gel using an SDS-PAGE system (Tianneng Company, Shanghai, China). Anti-ferritin heavy chain 1 (Santa Cruz, USA) was used as the primary antibody. Goat Anti-Rabbit IgG/AP (Santa Cruz, USA) was used as the secondary antibody. The relative expression of ferritin in BMSCs was analyzed from the band intensity using the Quantity One software (Bio-Rad), with normalization to GAPDH (Cell Signaling Technology, USA) (dilution 1 : 1000).

2.6. Evaluation of the Intracellular Iron Concentration and Cytotoxicity of Fth1 Expression by CCK8 and Prussian Blue Iron Staining. In order to evaluate the concentration of supplemented iron that the BMSCs can tolerate, the viability and intracellular iron accumulation of the cells were qualitatively assessed with Cell Counting Kit-8 (CCK8) and Prussian blue iron staining after 48 h of exposure to a range of iron concentrations. To find out the optimum ferric ammonium citrate concentration cultured with Fth1-
BMSCs while preparing for in vitro MRI examination, we cultured 3, 4, 5, and 7 mmol/ml ferric ammonium citrate with Fth1-BMSCs in the preliminary experiment, and considering cytotoxic effects and iron concentrations, we chose 5 mmol/ml ferric ammonium citrate for the next step. Fifth- or sixth-passage Fth1-BMSCs were plated at a density of 10000 cells/well, cultured in 96-well plates with 5 mmol/ml ferric ammonium citrate for 24 h, and then cultured for further 48 h. The absorbance of Fth1-BMSCs at 450 nm was measured using the Epoch Multi-Volume spectrometer system (BioTek Instruments, Winooski, VT, USA) after incubation for 1 h/2 h. All experiments were independently performed 5 times.

The average intracellular iron accumulation and distribution in the BMSCs were qualitatively assessed with Prussian blue iron staining. Fifth- or sixth-passage Fth1-BMSCs grown on coverslips were washed with PBS, fixed with 4% paraformaldehyde for 20 min, and stained with Prussian blue for 20 min; the cell nuclei were counterstained with nuclear fast red solution for 10 min. The positive and blank controls contained 50 μg/ml SPIO-BMSCs and BMSCs, respectively. The coverslips were mounted and examined under a microscope (Olympus BX51).

2.7. In Vitro MRI of Fth1-BMSCs. Fifth- to sixth-passage Fth1-BMSCs cultured in plates with an optimum concentration of ferric ammonium citrate for 24 h were washed with PBS to remove excess ferric ammonium citrate. Fth1-BMSCs (1 × 10^6 cells) were embedded in 600 μL PBS and 400 μL agarose (1%) uniformly in 1.5 mL cryotubes and kept for 30 min at room temperature (25°C). The positive and blank controls contained 50 μg/ml SPIO-BMSCs and BMSCs, respectively. We chose a series of survey images as observation indices including T2-weighted imaging (T2WI) and susceptibility-weighted imaging (SWI) sequences using a 1.5 T MRI scanner (General Electric, USA). Scanning parameters were as follows: (1) T2WI a fast spin echo (FSE) sequence, TR/TE = 2250/102 ms, flip angle = 25°, and depth = 2 mm. (2) SWI with a SWAN sequence, TR/TE = 87.2/44.3 ms, NEX = 1, flip angle = 15°, and depth = 1 mm. (3) DWI with a DW-EPI sequence, TR/TE = 3200/85.9 ms, b value = 0 and 800 s/mm^2, NEX = 4, and depth = 2 mm.

2.9. Histology and Immunohistochemistry. To confirm transgene expression after cell transplantation, animals were euthanized upon completion of the MRI scan, and the brains were removed and assessed by Prussian blue staining.

3. Results and Discussion

3.1. Culture and Identification of BMSCs. Flow cytometry was used to evaluate the purity of BMSCs that were extracted using the adherence method from total bone marrow cells: the percentage of cells that were positive for surface antigens CD44 and CD90 was 99.9% and 99.6%, respectively (n ≥ 3). There were only 5.5% cells expressing CD34, which showed that the purity of isolated BMSCs was high (Supplemental Figure 2).

Osteogenic induction culture: around days 12–14, cells exhibited a long spindle-shaped appearance, and scattered nodules could be observed. Alizarin Red staining was carried out on day 21 of induction, and orange-red-stained nodules could be seen (Supplemental Figure 3A).

Adipogenic induction: around day 12, some cells exhibit scattered lipid droplets and rounded cell morphology. After culturing for 21 d, cells were stained with Oil Red O, and many reddish-brown-stained mature adipocytes and immature adipocytes could be seen (Supplemental Figure 3B).

3.2. Morphological Observation of Fth1-BMSCs. Using a bicistronic lentiviral vector containing a fluorescence reporter [9, 15], we were able to confirm the successful integration of the transgenes via sequence analysis. This ensured constitutive expression and translation of the reporter proteins [16, 17]. Because of slow metabolism, the expression of EGFP was slow. The transduction efficiency was assessed after 3-4 days. Hyperintense spots were observed in cells that expressed EGFP using fluorescence microscopy (Supplemental Figure 4). The transduction efficiency was about 80%. The cells grew well and had normal morphology.
3.3. Expression of Reporter Gene Fth1 Assessed by Fluorescence Quantitative Real-Time PCR. After BMSCs were transfected by Fth1 lentivirus, fluorescence quantitative real-time PCR was used to assess the expression of the reporter gene Fth1, by measuring the amount of mRNA transcripts. qRT-PCR data showed that compared to the control group, in the EGFP-Fth1 group, Fth1 expression appeared significantly upregulated (Table 1). The FTH1 protein expression in BMSCs 7 d after transfection with the Fth1 lentivirus and in normal BMSCs (control group) was assessed by western blotting. The results showed that compared to the control group, FTH1 protein expression in the Fth1-BMSC group appeared significantly upregulated (Supplemental Figure 5).

3.4. Western Blot to Assess the Protein Levels from Translated mRNA. The FTH1 protein expression of BMSCs 7 d after transfection with the Fth1 lentivirus and normal BMSCs (control group) was all assessed by western blotting. The results showed that compared to the control group, FTH1 protein expression in the Fth1-BMSC group appeared to be upregulated significantly (Figure 1).

3.5. Evaluation of the Cytotoxicity of Fth1 Expression in BMSCs. The CCK8 method showed that the metabolic activity of the Fth1-BMSC group was higher than that of the control group (P < 0.05) (Table 2) (Figure 2).

3.6. Intracellular Iron Content in Fth1-BMSCs. Fth1-BMSCs cultured without ferric ammonium citrate were negative for Prussian blue iron staining. Fth1-BMSCs cultured with 5 mmol/ml ferric ammonium citrate showed blue masses, indicating that Fth1-BMSCs had a good iron uptake and storage capacity (Figure 3).

3.7. In Vitro MRI of Fth1-BMSCs. We observed that the Fth1-BMSC suspension showed a good signal contrast in the SWI series but had poor signal contrast in the DWI and T2WI series. Therefore, we chose the SWI sequence to detect the signal changes in the 50 μg/ml SPIO-BMSC and Fth1-BMSC groups. The 50 μg/ml SPIO-BMSC and Fth1-BMSC groups were significantly hypointense (Figure 4).

3.8. In Vivo MRI of Fth1-BMSCs and SPIO-BMSCs. After 50 μg/ml SPIO-BMSCs (control group) were injected in the direction of the cranium through the internal jugular vein, we observed well-defined lesions with low signal intensities in the T2WI series around the lateral ventricles of rats after 10 d; the tracing time was about 20 d. Fth1-BMSCs were injected in the same way. The MRI measurements were done 1 d, 10 d, 20 d, 40 d, and 60 d after transplantation. No obvious hypointense signal was observed on day 1 but appeared on the right side of the cortex in the DWI and SWI series on day 10. The hypointense signal migrated along nerve fiber tracts to the right side of the infarction lesion in the corpus striatum, representing the chemotaxis and migration of BMSCs. The hypointense signal was steady up to day 60 (Figure 5).

3.9. Histology and Immunohistochemistry. The rats were sacrificed after MRI in 60 days using an anesthetic overdose, and the brain was removed; contiguous coronal 12 μm thick

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### Table 1: Fluorescence quantitative real-time PCR results of control and experimental groups.

| Sample description | β-actin | Fth1 | ΔCT | ΔΔCT | 2^−ΔΔCT |
|--------------------|--------|------|-----|------|---------|
| Control            | 18.52  | 28.01| 9.49| 0    | 1       |
| Fth1               | 18.12  | 23.49| 5.37| −4.11| 17.34   |

Note: the quantitative assay of each sample was conducted thrice; using internal parameters, the relative quantities of mRNA were calculated using the $2^{-\Delta\Delta CT}$ algorithm. $2^{-\Delta\Delta CT}$ represents the consequence. By the standard of cells responding to EGFP-Fth1 viral infection, $2^{-\Delta\Delta CT}$ was calculated.

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### Table 2: Absorbance values of experimental and control groups 48 h after transfection.

| Group       | OD value (x ± s) | p value |
|-------------|------------------|---------|
| Control     | 1.2289 ± 0.0367  | <0.05   |
| Fth1-BMSC   | 1.2821 ± 0.0253  | <0.05   |

Note: OD: optical density; $p < 0.05$, if there are statistical differences.
sections were prepared for Prussian blue staining. Prussian blue staining was seen on the right side of infarction lesion (Figure 6).

3.10. Discussion. We have generated mesenchymal stem cells expressing Fth1 or labeled with SPIO with the aim of exploring how these iron markers can impact T2WI and SWI contrast using BMSCs. We showed that though we chose a high dose of SPIO ($6 \times 10^4$/ml) [18], the T2 relaxation time became progressively shorter during in vivo MRI tracking of SPIO-BMSCs implanted in the rat cerebral infarcted area probably because of cell mitosis leading to a decrease in the SPIO concentration with each division [8] and because of macrophage phagocytosis, which clears necrotic cells, including SPIO-labeled cells. Since SPIO cells were undetectable in the infarction lesion, we could only perform a pathological analysis. Moreover, the existing technique was too insensitive for tracing low concentrations of iron (SPIO), which affected and limited long-term observation of the biological behavior of BMSCs. Therefore, we used BMSCs transfected with the Fth1 gene using a lentivirus. The Fth1-BMSCs were transplanted to treat focal cerebral infarction induced in rats, and the sensitivity and

Figure 3: Efficiency of Prussian blue staining in (a, c) BMSCs (x100), where no positively stained cells were seen; (b) Fth1-BMSCs (x100), where positively stained cells showed blue-colored masses; (d) SPIO-BMSCs (x100), where positively stained cells showed blue-colored masses.

Figure 4: Measurement of cellular iron content and in vitro MRI analysis. MRI images of (a) SPIO-BMSCs showing circular hypointense regions, (b) Fth1-BMSCs showing circular hypointense regions, and (c) the control group without obvious hypointense regions.
time duration of signal changes in different MRI sequences were observed, to identify a suitable labeling method for the long-term monitoring of the migration and proliferation of mesenchymal stromal cells.

Ferritin is a ubiquitously expressed intracellular iron-storage protein, keeping iron in a soluble and nontoxic form [19, 20]. The ferritin heavy chain has ferroxidase activity and is responsible for the upregulation of the transferrin...
receptor, which increases iron uptake. Ferritin heavy chain expression levels showed a linear correlation with the T2 relaxation time [21–23]. *Fth1* can be used as an endogenous reporter gene; when introduced into a lentiviral vector and then used to establish a clonal transgenic BMSC line, the reporter gene can be steadily inherited by the daughter cells during mitosis, indicated by the stable hypointensity observed by MRI [24, 25]. Thus, the *Fth1*-labeling method may be an effective method for long-term monitoring of the biological activity of cells by MRI.

Naumova et al. [16] reported a systematic evaluation of ferritin-labeled stem cells in infarcted mouse hearts *in vivo*, using three cardiac-gated pulse sequences in a 3T scanner. The graft size measurements by T2 iMSDE (improved motion-sensitized driven-equilibrium) and T2 GRE (gradient recalled echo) were highly correlated with histological assessments. These data supported the use of ferritin to track the survival, growth, and migration of stem cells transplanted into the injured heart. Iordanova [17] described an efficient ferritin-based MRI reporter and its use in labeling mouse subventricular-zone progenitors, enabling *in vivo* visualization of endogenous neuroblast migration toward the olfactory bulb. This MRI reporter gene platform can facilitate the noninvasive study of the migration of native or transplanted stem cells and the associated neurogenic or therapeutic molecular events in live animals.

In our *in vitro* experiments, we found that when *Fth1*-BMSCs were cultured in the complete medium, they were well detected by MRI. The ferric ammonium citrate had two main characteristics: first, it assured that *Fth1*-BMSCs could be overexpressed and thus augment iron uptake to reach the detectable threshold of MRI *in vitro*; second, the toxicity of ferric ammonium citrate on cell proliferation could be reduced.

Previous studies have rarely addressed whether FTH1 can be successfully detected by MRI after administration of the *Fth1*-BMSCs and whether *Fth1*-BMSCs can reach the infarction lesions in mouse stroke models when injected through the internal jugular vein [26, 27]. In our *in vivo* experiments, the lacunar stroke model was induced by middle cerebral occlusion. We set up a normal control group, a sham group, and an operation group, and internal jugular vein injection was used to transplant SPIO-labeled BMSCs or *Fth1*-labeled BMSCs. MRI detected BMSCs in the *in vivo* images of different groups. Our present results demonstrate that the SPIO-BMSCs and *Fth1*-BMSCs transplanted by internal jugular vein injection after cerebral ischemia reperfusion injury can be detected *in vivo* and result in significant hypointense signals around the infarction lesion [28]. Statistical analysis showed that SPIO-BMSCs showed lower transverse relaxation and distribution compared to *Fth1*-BMSCs, and the reason needs further exploration. A comparison of the extent of iron aggregation in the infarction lesions showed that *Fth1*-BMSCs accumulated more iron than SPIO-BMSCs under stroke condition. This was reflected in the SWI sequence *in vivo*, which

![Prussian blue staining of rat brains: positively stained cells showed blue-colored masses. (a, b) Fth1-BMSCs (×200). (c, d) SPIO-BMSCs (×200).](image-url)
showed a lower signal contrast compared to the control group. Prussian blue staining and pathological observation showed that a high density of blue-stained iron particles accumulated around the infarction area and grew into the infarction center in the \textit{Fth1}-BMSCs group, which suggested that \textit{Fth1}-labeled BMSCs could take up iron particles from the surrounding tissue.

SWI clearly showed hypointense lesions in the SPIO-BMSC group (only traceable for 30 d) and the \textit{Fth1}-BMSC group. T2WI showed hypointense lesions for only 20 d in the SPIO-BMSC group. T2WI was not sensitive enough for the \textit{Fth1}-BMSC group. This indicated that the intracellular iron concentration of \textit{Fth1}-labeled BMSCs was equivalent to the intracellular iron concentration of the 50 \textmu g/ml SPIO-labeled BMSCs on the 20\textsuperscript{th} day. Compared to the T2WI sequences, SWI sequences show a longer and a wider range of hypointense signals. Therefore, SWI is more sensitive and can be chosen as the optimal MRI-tracking sequence.

\textit{Fth1}-BMSCs maintain the same signal intensity between the 10\textsuperscript{th} and the 60\textsuperscript{th} day and show a similar distribution range between the 40\textsuperscript{th} and the 60\textsuperscript{th} day. Although current medical imaging technologies restricted us from detecting very low concentrations of iron, we can exclude that the \textit{Fth1}-BMSCs multiplied and migrated around the lesion; that is, mitosis of BMSCs may have stopped, and committed differentiation might have occurred. Hence, it is possible to observe the proliferation and differentiation of stem cells by the \textit{Fth1}-labeling method \cite{15, 27, 29}. The \textit{Fth1}-labeling method was stable and could be used for long-term tracking.

4. Conclusions

In summary, \textit{Fth1} coupled to a shuttle plasmid (pCDH-CMV-MCS-EF1-\textit{copGFP}) displays satisfactory properties for utilization as an MRI reporter gene for \textit{in vivo} detection of BMSCs transplanted in response to cerebral ischemia reperfusion injury and used for therapeutic interventions. The \textit{Fth1}-labeling method, based on a standard clinical MRI, can potentially prove to be a powerful tool for the combined, noninvasive evaluation of molecular activity of transplanted cells and is stable and suitable for long-term tracking of cells. Compared to T2WI, SWI is more sensitive and can be chosen as the optimal MRI-tracking sequence.

In this study, we provided a proof of the concept that a molecular probe constituting the \textit{FTH} gene can be utilized for the detection of the chemotaxis and migration of BMSCs by MRI. In addition, we could also document a marked beneficial effect of \textit{Fth1}-BMSC administration; however, specifically designed studies will be necessary to further characterize the therapeutic actions of BMSCs on cerebral ischemia reperfusion injury models. Further work will be necessary to test the reliability of this methodology for tracking changes over the entire process of postinfarct remodeling and under other physiological and pathological conditions. In addition, more research is required to study the long-term safety and differentiation properties of \textit{Fth1}-BMSCs in rat models.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethical Approval

All interventions and animal care procedures were performed in accordance with the Laboratory Animal Welfare Act, Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD), and Guidelines and Policies for Animal Surgery provided by our hospital (Shanghai Jiao Tong University, Shanghai, China) and were approved by the Institutional Animal Use and Care Committee.

Disclosure

The funders do not have any role in the design of the study, collection, analysis, and interpretation of data, and writing of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

HXL wrote the manuscript. ZJM revised the manuscript. XY and WJL performed the statistical analyses. ZQ analyzed the tables. HXL and ZJM collected the data and are responsible for the integrity of the registered data. HXL and ZJM designed the study and contributed to the analysis and interpretation of data. All authors read and approved the final manuscript.

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Supplementary Materials

Supplemental Figure 1: vector plasmid profile and multiple cloning site information of (A) pCDH-CMV-MCS-EF1-copGFP-\textit{Fth1}, (B) psPAX2, and (C) pMD2.G. Supplemental Figure 2: results of BMSC surface antigens CD90, CD44, and CD34 detected by flow cytometry. Supplemental Figure 3: results of staining with Alizarin Red and Oil Red O 21 d after osteoblastic (A) and adipogenic (B) induction of BMSCs. Supplemental Figure 4: results of fluorescent microscopy after EGFP gene expression in \textit{Fth1}-BMSCs for 48 h and further culturing for 3-4 d (A) in normal light and (B) in fluorescent light). Supplemental Figure 5: results of \textit{FTH1}}
protein expression in the control group and Fth1-BMSC group. (Supplementary Materials)

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