In vivo Imaging of Tumor Transduced with Bimodal Lentiviral Vector Encoding Human Ferritin and Green Fluorescent Protein on a 1.5T Clinical Magnetic Resonance Scanner

Hoe Suk Kim1,2, Hye Rim Cho1, Seung Hong Choi1, Ji Su Woo1, and Woo Kyung Moon1,2

Abstract
A combination of reporter genes for magnetic resonance imaging (MRI) and optical imaging can provide an additional level of noninvasive and quantitative information about biological processes occurring in deep tissues. We developed a bimodal lentiviral vector to monitor deep tissue events using MRI to detect myc-tagged human ferritin heavy chain (myc-hFTH) expression and fluorescence imaging to detect green fluorescent protein (GFP) expression. The transgene construct was stably transfected into MCF-7 and F-98 cells. After transplantation of the cells expressing myc-hFTH and GFP into mice or rats, serial MRI and fluorescence imaging were performed with a human wrist coil on a 1.5T MR scanner and optical imaging analyzer for 4 weeks. No cellular toxicity due to overexpression of myc-hFTH and GFP was observed in MTT and trypan blue exclusion assays. Iron accumulation was observed in myc-hFTH cells and tumors by Prussian blue staining and iron binding assays. The myc-hFTH cells and tumors had significantly lower signal intensities in T2-weighted MRI than mock-transfected controls (P ≤ 0.05). This is direct evidence that myc-hFTH expression can be visualized noninvasively with a 1.5T clinical MR scanner. This study shows that MRI and fluorescence imaging of transplanted cells at molecular and cellular levels can be performed simultaneously using our bimodal lentiviral vector system. Our techniques can be used to monitor tumor growth, metastasis, and regression during cell and gene-based therapy in deep tissues. Cancer Res; 70(18); 7315–24. ©2010 AACR.

Introduction
Fluorescent or bioluminescent proteins have been used as reporter genes for noninvasive imaging of in vivo events, which can be used to assess the efficacy of gene therapy and metabolic activity (1), growth kinetics of transformed tumor cells (2, 3), and differentiation of stem cells (4, 5). However, fluorescence reporters are most useful for visualization of peripheral organs like skin, due to their limited ability to provide spatial resolution and anatomic information in deeper tissues, which is critical for visualization of events such as tumor metastases that occur throughout the body. However, combining fluorescent reporter genes with iron-accumulating reporter genes for magnetic resonance imaging (MRI) provides noninvasive, high-resolution, and quantitative information for biological processes occurring in deep tissues.

The main advantage of using iron-accumulating reporter genes for MRI is that it overcomes the challenge of delivering the contrast agent to deep tissues (6). Recently, the ferritin gene has been used as an iron-accumulating reporter gene for evaluating the efficacy of gene and cell therapy by MRI (7–12). Ferritin is an iron storage protein that can be ectopically expressed to augment endogenous iron uptake and produce signal changes in the surrounding environment that can be detected by MRI. Ferritin-based imaging with high magnetic field magnetic resonance (MR) scanner, such as 4.7T or 7T scanner, has been performed for cells and tissues transfected with plasmid carrying the ferritin gene (9, 11, 12). The low MRI contrast and signal intensities in these studies are likely to be due to the inherent limits of gene delivery by plasmid. Therefore, the development of a suitable vector for MR reporter gene delivery is a major goal that must be reached to produce detectable signal and contrast for clinical MR applications.

Reportedly, lentiviral vectors derived from HIV type 1 have efficient delivery, integration, and long-term and stable expression of transgenes for mitotic and nonmitotic cells both in vivo (13, 14). Furthermore, in contrast to adenoviral vectors, lentiviral vector delivery systems do not produce humoral responses to injected viral antigens (15). These critical features of lentiviral vector delivery have made them the logical choice for gene therapy treatment of cancer or diseases with a genetic basis (14, 16, 17). We believe that the generation of safe and efficacious lentiviral vectors will significantly

Note: H.S. Kim and H.R. Cho contributed equally to this work.

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advance the prospects of multimodal imaging technique using 1.5T MR scanners.

Here, we developed a lentiviral vector construct that expresses both recombinant human ferritin [myc-tagged human ferritin heavy chain (myc-hFTH)] and green fluorescent protein (GFP) for simultaneous MRI and fluorescence imaging in vitro and in vivo. In our system, myc-hFTH cells and tumors had more iron accumulation than mock-transfected controls, which produced a pronounced increase in T2* contrast effects on a 1.5T clinical MR scanner. Our lentiviral myc-hFTH vector system overcomes the present limitations of reporter gene delivery for MRI by providing direct, strong, and noninvasive imaging of deep tissues in vivo using a 1.5T clinical MR scanner.

Materials and Methods

Construction of dual-promoter lentiviral vectors encoding hFTH and GFP

The recombinant lentiviral vector (LentiM1.41) was constructed in the laboratory of Yeon-Soo Kim (Inje University, Gimhae, South Korea). The phosphoglycerate kinase promoter was inserted immediately upstream of GFP in the lentiviral vector into a BamHI-EcoRI site available for insertion of a gene of interest under the control of the cytomegalovirus (CMV) immediate-early promoter and puromycin-resistant gene by the IRES system. Briefly, the hFTH gene was amplified from pOBT7-human FTH (BC073750) by PCR with the primers forward, BamHI 5′-CGGGATCCGCCACCATGGAACAAAAACTCATCTCAGAA-GAGGATCTGATGACGACCGCGTCCACC-3′ and reverse, EcoRI 5′-CGGAATTCGCCACCATGGAACAAAAACTCATCTCAGAA-GAGGATCTGATGACGACCGCGTCCACC-3′, followed by digestion with BamHI and EcoRI and insertion in the restriction sites downstream of the CMV promoter. The NH2 terminus of hFTH was tagged at the NH2 terminus with the mouse c-myc gene to produce recombinant myc-hFTH. The sequence of the cloned cDNA was confirmed by DNA sequencing. The constructed lentiviral vector expressing myc-hFTH was named Lentimyc-hFTH (Fig. 1A). Lentiviral vector without GFP and hFTH was used for control (mock) transfections.

Lentivirus production

The recombinant lentivirus was produced by Macrogen Lentivirus Institute. Briefly, three plasmids, a transfer vector, a VSV-G expression vector, and a LentiVector Institute. Briefly, three plasmids, a transfer vector, a VSV-G expression vector, and a gag-pol expression vector, were cotransfected into 293T cells at a 1:1:1 molar ratio. The culture supernatant containing viral vector particles was harvested 48 hours after transfection, clarified with a 0.45-μm membrane filter (Nalgene), and immediately stored at −70°C. Titers were determined by p24 ELISA or infection of HeLa cells. The GFP expression of transduced cells was observed and imaged with a fluorescence microscope and camera system. In routine preparations, the unconcentrated titer was ~10^6 to 10^7 transduction units/mL.

Generation of MCF-7 and F-98 cells stably expressing myc-hFTH and GFP

Human breast cancer cells (MCF-7) and rat glioma cells (F-98) were obtained from American Type Culture Collection, cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and passaged in our laboratory for <6 months after resuscitation. MCF-7 cells expressed the estrogen receptors. F-98 cells provided a reproducible tumor model when inoculated into the brains or under the skin of Fisher 344 rats.

For stable cell generation, MCF-7 and F-98 cells were transduced with lentivirus supernatants for 6 to 10 hours in the presence of 4 to 8 μg/mL polybrene. Transduced cells were selected using 1 μg/mL puromycin in the media for 3 to 4 weeks and sorted using fluorescence-activated cell sorter. myc-FTH and GFP expressions in all clones were analyzed by reverse transcription-PCR (RT-PCR), Western blot, immunostaining, and fluorescence microscopy (Nikon). Clones that express high levels of myc-hFTH and GFP were passaged and maintained in a growth medium containing 1 μg/mL puromycin. To further confirm the stable expression of myc-hFTH and GFP of selected clones, Western blot and immunostaining were performed before in vivo and in vitro experiments.

Fluorescence-activated cell sorting analysis

Fluorescence of GFP in transduced cells was analyzed using a FACSCalibur flow cytometer (BD Biosciences) equipped with a 530-nm filter (bandwidth, ±15 nm), a 585-nm filter (bandwidth, ±21 nm), and a CellQuest software (BD Biosciences). Sorted GFP-expressing cells were used for in vivo and in vitro studies.

RT-PCR

Transduced MCF-7 and F-98 cells were selected by puromycin (2.5 μg/mL) resistance. Human FTH mRNA expression was detected by RT-PCR analysis. RNA was isolated from confluent 10-cm culture plates using the TRIzol reagent (Invitrogen), and RT-PCR was performed using the Superscript III One-Step RT-PCR kit (Invitrogen) according to the manufacturer’s protocol.

Western blot

myc-hFTH protein levels were evaluated by Western blot analysis. The cells were washed with cold PBS and lysed with NP40 lysis buffer including protease inhibitors (0.1 mmol/L phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, 5 μg/mL pepstatin A, and 1 μg/mL chymostatin). The lystate protein concentration was evaluated with the bicinchoninic acid method (Pierce Biotechnology), and ∼30 μg of protein were loaded per lane on a polyacrylamide denaturing gel for electrophoresis. Protein was transferred to nitrocellulose membranes for blotting. The antibodies used were rabbit polyclonal antibody to FTH (Santa Cruz Biotechnology), mouse monoclonal antibody to myc (Santa Cruz Biotechnology), rabbit polyclonal antibody to GFP (Santa Cruz Biotechnology), and mouse monoclonal antibody to β-actin for loading controls (Sigma). Primary antibodies were detected by horseradish peroxidase–conjugated goat anti-mouse or anti-rabbit IgG (Santa Cruz Biotechnology).

Assessment of iron accumulation

For iron loading, the cells were grown in supplemented medium that included ferric ammonium citrate (FAC;
20–500 μmol/L) for 72 to 98 hours. Briefly, cells were cultured in a medium supplemented with increasing concentrations of FAC, washed three times with PBS, resuspended in 6 N HCl, and incubated at 70°C for 30 minutes. The iron amount was determined using a total iron reagent kit (Pointe Scientific). Average iron loadings were calculated by dividing the total mean values by total protein amounts. Prussian blue iron staining was used to assay for iron accumulation. Cells were grown on eight-well chamber slides in a medium with or without FAC for 72 to 98 hours. The cells were washed thoroughly in PBS and fixed in 4% paraformaldehyde for 10 minutes before staining. The eight-well chamber slides were placed in a staining solution (1% potassium ferrocyanide and 5% HCl) for 30 minutes, washed twice in PBS, counterstained with fast red, and mounted on slides with a water-soluble mounting medium (Gel/Mount; Biomeda Corp.). Prussian blue staining for iron in tumor sections was performed as described above.

**Immunofluorescence**

Cells were cultured on eight-well chamber slides and rinsed in PBS before fixation in 2% paraformaldehyde in PBS for 30 minutes. Fixed cells were incubated with primary antibodies directed against myc and GFP in cells, and staining was visualized using Alexa 594–conjugated and Alexa 488–conjugated secondary antibodies (Invitrogen). After s.c. transplantation of MCF-7 or F-98 cells, tumors were collected at 2 to 4 weeks and fixed in 10% formalin. Prepared paraffin sections (4-μm thickness) were dewaxed, hydrated, and treated with 0.01% protease XXIV (Sigma) in PBS for 20 minutes at 37°C. Sections were then incubated with primary antibodies to myc and GFP according to the manufacturer’s instructions. myc-hFTH and GFP were acquired in myc-hFTH–transduced cells. D, Western blots for myc-hFTH and GFP using anti-myc, anti-FTH, and anti-GFP antibodies.
GFP were visualized with Alexa 549–conjugated and Alexa 488–conjugated secondary antibodies.

**Cell toxicity assay**

To determine cell viabilities and proliferative abilities, cells were initially seeded in 96-well plates at $10^4$ per well. Cell viability was assessed using a standard MTT assay. Cell proliferation was evaluated using trypan blue exclusion assays. The proliferative activities were expressed as the relative percentage of cell numbers at day 0.

**Determination of the mitochondrial membrane potential**

Changes in the mitochondrial membrane potential of cells cultivated in an iron-rich medium treated with FAC were determined with the vital mitochondrial dye JC-1 (Molecular Probes). Cells were grown in an eight-well chamber tissue culture dish. The cells were either treated with 100 μmol/L FAC or untreated for 72 hours. After treatment, the medium was replaced with a serum-free medium containing 10 μg/mL JC-1, a potential-dependent J-1 aggregate-forming lipophilic cation. Cells were incubated at 37°C for 10 minutes, followed by washing with PBS. Immediately, the cells were visualized by a confocal laser scanning microscope (Leica SP2). The monomer and JC-1 aggregate forms were simultaneously excited by a 488-nm argon laser sources (18). Mitochondrial depolarization was marked by punctate orange-red fluorescence.

**Tumor model**

To evaluate the efficacy of the myc-hFTH gene imaging approach *in vivo*, MCF-7 and F-98 cells transduced with a
Lenti-myc-hFTH vector or a control vector were transplanted s.c. into mice or rats. MCF-7 cells (2 × 10⁷) were suspended with Matrigel and injected s.c. into the shoulders of 6-week-old BALB/c nude mice (n = 2). Mice were imaged at 2, 7, and 21 days after transplantation. To further evaluate rat tumor models, F-98 cells (1 × 10⁶) were suspended in PBS and injected s.c. into the shoulders of 6-week-old Fisher 344 rats (n = 9), and the rats were imaged at 1, 2, 3, and 4 weeks after transplantation.

**Fluorescence imaging**

All fluorescence imaging was performed using the fluorescence imaging system from Maestro Cambridge Research and Instrumentation. For *in vitro* fluorescence imaging, the collected cells were prepared in 1.5-mL centrifuge microtubes and imaged using excitation at 470 nm and emission at 535 nm. Whole-body *in vivo* and *ex vivo* tumor imaging was performed in a fluorescent light box illuminated by fiber optic lighting at 465 nm.

**MRI**

To evaluate the detection sensitivity by FTH overexpression compared with other methodologies involving uptake of Feridex (Advanced Magnetics), the T₂* relaxation times on T₂*-weighted MRI of phantoms consisting of 2 × 10⁵, 2 × 10⁶, 2 × 10⁷, and 2 × 10⁸ cells were measured in mock, myc-hFTH, and Feridex-labeled cells. Feridex is a clinically approved and commercially available superparamagnetic iron oxide (SPIO) contrast agent. Feridex-labeled cells were collected after 24 hours of incubation with 12.5 μg Fe/mL Feridex. To prepare an MR phantom, mock, myc-hFTH, myc-hFTH + FAC, and Feridex-labeled cells were suspended in 1% agarose gel and transferred into 1.5-mL centrifuge microtubes. *In vitro* and *in vivo* T₂*-weighted images of phantoms and tumors at different times after the transplantation were acquired on the 1.5T MR scanner with a wrist coil or a head coil (GE Signa Excite). A conventional CPMG sequence with 12 multiple TEs (TR/TE = 3300 ms/13, 26, 39, 52, 70, 140, 210, 280, 400, 800, 1200, and 1600 ms) for T₂* measurements was performed. The measurement parameters were as follows: TR = 3400 ms, TE = 100 ms, FOV = 60 × 60 mm², ETL = 16, matrix = 256 × 256, slice thickness/gap = 2.0 mm/0 mm, and NEX = 2.0.

For analysis of MRI data, T₂* relaxation times were calculated by fitting the signal intensities (SI) with increasing TEs. The mean SI within the region of interest was also computed using a standard MRI operating system software program. The MRI SI data were normalized using the mean signal score derived from the muscle. The ratios of SI changes for tumor versus muscle on T₂-weighted images were calculated according to the formula SI = ([SI]tumor/[SI]muscle).

**Statistical analysis**

Data were presented as means ± SDs of more than three independent experiments. Comparisons were performed using the Student’s *t* test. Differences were considered significant at *P* values of ≤0.05.

**Results**

**Expression of myc-hFTH and GFP in cancer cells**

The dual-promoter lentiviral vector was successfully constructed to express myc-hFTH and GFP proteins (Fig. 1A). The transduction efficiency of MCF-7 and F-98 cells expressing GFP abundantly was >90% using the Lenti-myc-hFTH vector (Fig. 1B). We used FACS-isolated, GFP-positive cells...
for all experiments. We confirmed the presence of myc-hFTH and GFP expression in transfected cells and their absence in the no-vector and mock controls using immunofluorescence and Western blot (Fig. 1C and D).

**Increased iron accumulation in cells expressing myc-hFTH**

We investigated if myc-hFTH overexpression with lentivirus influenced cell viability and growth. No differences in cell viability and growth were observed between the myc-hFTH, mock, and no-vector cells (Fig. 2A and B). We further investigated the viability and mitochondrial membrane potential of myc-hFTH cells after iron overload by treatment with FAC. Iron is a cofactor for enzymes involved in many metabolic processes, but free iron can also be harmful, because its excess is known to enhance the production of reactive oxygen species and induce cell injury/apoptosis via a mitochondrial permeability transition (19). The most widely implemented application of JC-1 is for the detection of mitochondrial depolarization occurring in the early stages of apoptosis. The dye undergoes a reversible change in fluorescence emission from green to orange-red as mitochondrial membrane potential increases. Therefore, the orange-red/green ratio can help to identify the viability status of a cell. As shown in Fig. 2C, no-vector, mock, and myc-hFTH normal cells untreated with FAC or H2O2 (Fig. 2C) fluoresced green. However, all cells treated with 100 μmol/L H2O2 for 3 hours fluoresced orange-red, which indicated mitochondrial potential collapse. The treatment with 100 μmol/L FAC caused higher mitochondrial polarization in no-vector and mock
cells than in myc-hFTH cells (Fig. 2C). The treatment with FAC (20–500 μmol/L) for 72 hours did not cause cellular toxicity in myc-hFTH cells, whereas the viability of no-vector and mock cells decreased after treatment with FAC (Fig. 2D). The results imply that myc-hFTH gene expression was protective against FAC toxicity. Iron accumulation of all cells was increased in a dose-dependent manner by treatment with FAC (20 and 500 μmol/L). myc-hFTH cells had more accumulation of intracellular iron than mock and no-vector cells (Fig. 2E). As shown in Fig. 2F, the accumulated iron of myc-hFTH cells cultivated in a medium supplemented with FAC (500 μmol/L) was detected by Prussian blue stain.

**In vitro MRI and fluorescence imaging of myc-hFTH cells**

T2*-weighted MRI and fluorescence imaging of the agarose phantom containing 1 × 10^7 mock and myc-hFTH cells treated with or without FAC were performed on a fluorescence imaging system and a 1.5T MR scanner. GFP fluorescence imaging was obtained from the agarose phantom containing myc-hFTH cells (Fig. 3A). The dark signal was easily discernable in myc-hFTH cells treated with FAC (300 μmol/L; Fig. 3B). T2* relaxation times of mock and myc-hFTH cells treated without FAC were 30.3 ± 6.5 and 26.01 ± 1.4 ms, respectively (Fig. 3C). T2* relaxation times of mock and myc-hFTH cells in the presence of FAC were significantly lower, 22.03 ± 1.14 ms and 18.3 ± 0.31 ms, respectively (Fig. 3C).

In detection sensitivity studies, as the number of cells increased, the T2* relaxation times on T2*-weighted MRI of phantoms and all groups (mock, myc-hFTH, and Feridex-labeled cells) gradually decreased (Fig. 3D). The signal drops on T2*-weighted images were not observed for the 2 × 10^2 myc-hFTH cells and Feridex-labeled cells compared with mock cells. However, we could detect significant signal drops for 2 × 10^3 myc-hFTH cells and Feridex-labeled cells compared with the same number of mock cells. T2* relaxations of 2 × 10^3 mock, myc-hFTH, myc-hFTH + FAC, and Feridex-labeled cells were 444.4 ± 96.2 ms, 305.6 ± 48.1 ms, 277.8 ± 48.1 ms, and 169.8 ± 28.7 ms, respectively. T2* relaxations of 2 × 10^3 mock, myc-hFTH, myc-hFTH + FAC, and Feridex-labeled cells were 444.4 ± 96.3 ms, 241.3 ± 103.8 ms, 188.9 ± 19.24.1 ms, and 144.8 ± 20.9 ms, respectively. The detection sensitivity of in vitro MRI of Feridex-labeled cells was greater than that of myc-hFTH cells (P < 0.05).

**In vivo MRI and fluorescence imaging of myc-hFTH tumors**

Twenty-one days after s.c. transplantation of MCF-7 cells, fluorescence imaging of GFP in myc-hFTH tumors in nude mice was detected, but not in mock tumors (Fig. 4A, left). We detected lower signals on T2-weighted images of myc-hFTH tumors than of the mock tumors on a 1.5T MR scanner (Fig. 4A, right).

Further evaluation was performed using MRI and fluorescence imaging of rat tumor models. A hypointense signal in T2- and T2*-weighted images of myc-hFTH tumors was detected (Fig. 4B, left). T2* relaxations times of mock and myc-hFTH at 3 weeks after transplantation were ~63.3 ± 7.5 ms and 35.9 ± 6.1 ms, respectively (Fig. 4B, right). T2* values of myc-hFTH tumors were significantly lower than...
Table 1. Summary of in vivo MRI using endogenous Ferritin reporters

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Discussion

Of all the imaging modalities, MRI provides the highest spatial resolution and the most anatomic and physiologic information in deep tissues, which makes it readily translatable to clinical applications (20, 21). Multimodality imaging is widely considered to involve the incorporation of two or more imaging modalities, usually within the setting of a single examination, using optical studies, nuclear medicine, computed tomography, or MRI (22–24). Noninvasive multimodality imaging techniques have emerged as an essential tool for imaging of the exact localization, extent, and metabolic activity of the target tissue.

Other groups have shown that in vitro and in vivo MRI of SPIO-labeled single cells can be acquired using an optimized MR hardware and a 1.5T clinical MR scanner (25–27). However, MRI contrast agents can cause unpleasant side effects including toxic, allergic, and hypersensitivity reactions (28, 29). Furthermore, long-term retention of SPIO in dividing cells is a limitation of this approach (29). The loss of intracellular SPIO from dividing cells is attributed to the dilution due to cellular division or exocytosis. An advantage of using MRI reporter genes to introduce contrast agents is that long-term imaging is possible and the negative side effects of exogenous MR contrast agents are avoided. Ferritin, the iron-storing protein of mammals, can store up to 4,500 iron and has been accepted as a universal MRI reporter gene (7–12, 30). The presence of ferritin in organs influences the contrast of T2-weighted MRI at very low iron concentrations. However, recent studies for a visualization of cancer cells and tumor tissues using plasmid vector encoding ferritin has been performed on high magnetic field MR scanners (Table 1) and reported that native ferritin is a weak T2 contrast enhancing agent in practice. Despite this, Liu and colleagues recently showed that, when ferritin is introduced with lentiviral vector, it is useful for noninvasive MRI monitoring embryonic stem cells in vivo (12).

Lentiviral vectors have become attractive vehicles for gene transfer and expression because lentivirus can maintain target gene expression for up to 6 months. The ability of lentiviral vectors to integrate into both nondividing cells and terminally differentiated cells and to provide stable and long-term gene expression in vivo is a desirable attribute for many scientific approaches (17, 31). Lentiviral vector is the preferred method for stable integration of reporter genes. Therefore, we developed a lentiviral vector to transfer ferritin effectively into target cancer cells and tissues and to increase the ferritin expression level and duration for more effective contrast during MRI. Our viral vector system was designed to allow MRI and fluorescence imaging simultaneously. It had very high transduction efficiency and produced stable expression in cultured cells without decreasing viability or proliferation. We obtained dark signals and a significant decrease in T2* relaxation time from T2*-weighted images of GFP expressing tumors (Fig. 4D).

Histologic analysis of myc-hFTH tumors

Western blotting showed that myc-hFTH and GFP proteins were only expressed in myc-hFTH tumors (Fig. 5A). Immunostaining of mock and myc-hFTH tumors with anti-myc antibody and anti-GFP antibody was performed. The expression of GFP and myc-hFTH proteins in myc-hFTH tumors was detected, and the sites expressing myc-hFTH also expressed GFP (Fig. 5B). To verify the ability of myc-hFTH protein to augment iron storage, we performed Prussian blue staining to detect the accumulated iron in myc-hFTH tumors 3 weeks after transplantation. Large deposits of accumulated iron were detected in the myc-hFTH tumors (Fig. 5C).
that previously available ferritin reporters are sensitive enough for detection of cells and tissues using a 1.5T clinical MR scanner. Thus, our lentiviral vector system is the first ferritin reporter to provide sufficient sensitivity for the detection of 2 × 10^3 cells with a clinical MR scanner. Although we did not detect ferritin-transduced single cells in vitro or in vivo, our lentiviral vector was more effective for ferritin gene transfer and expression and for the detection of cancer cells and tumor tissues than previously described expression systems. This improvement in the sensitivity of ferritin-transduced cell detection by MRI is critical for clinical applications. MRI detection sensitivity can also be increased by combining ferritin heavy and light polypeptide chimera expression with iron supplementation (32, 33). Our future goals are to improve the MRI sensitivity for detecting single cells and small populations of cells by combining our lentiviral expression system with ferritin light polypeptide expression and iron supplementation. The development of improved MRI sensitivity on the single-cell level will facilitate progress in understanding the mechanisms of metastasis and the improvement of cancer therapeutics.

Ferritin is an antiapoptotic gene that protects cells and tissues against oxidative stress (34). Free iron is toxic because it facilitates the generation of highly reactive oxygen radical species that can damage cellular constituents. Balancing the deleterious and beneficial effects of iron is an essential aspect of cell survival (35). Overexpression of myc-hFTH protein had no deleterious effects on cell viability or the mitochondrial membrane potential of cells, although it increased iron storage. Our results imply that myc-hFTH can be safely used for the long-term tracking of transplanted cells and tissues by MRI, as well as for protection of transplanted cells and tissues against oxidative stress.

In summary, we have successfully simultaneously imaged myc-hFTH and GFP-transduced cells and tumors, both in vitro and in vivo, with a 1.5T clinical MR scanner and fluorescence imaging analyzer. This technology can be applied to monitor tumor growth, metastasis, and regression and multidrug cell and gene-based therapy in deep tissues.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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