Magnetic Resonance Imaging of Mesenchymal Stem Cells Homing to Pulmonary Metastases Using Biocompatible Magnetic Nanoparticles

Michael R. Loebinger,1 Panagiotis G. Kyrtatos,2,4 Mark Turmaine,3 Anthony N. Price,2 Quentin Pankhurst,4 Mark F. Lythgoe,2 and Sam M. Janes1

1Centre for Respiratory Research, Rayne Institute, 2Centre for Advanced Biomedical Imaging, Department of Medicine and Institute of Child Health, and 3Division of Biosciences, University College London; and 4Davy-Faraday Research Laboratory, The Royal Institute of Great Britain, London, United Kingdom

Abstract

The ability of mesenchymal stem cells (MSC) to specifically home to tumors has suggested their potential use as a delivery vehicle for cancer therapeutics. MSC integration into tumors has been shown in animal models using histopathologic techniques after animal sacrifice. Tracking the delivery and engraftment of MSCs into human tumors will need in vivo imaging techniques. We hypothesized that labeling MSCs with iron oxide nanoparticles would enable in vivo tracking with magnetic resonance imaging (MRI). Human MSCs were labeled in vitro with superparamagnetic iron oxide nanoparticles, with no effect on differentiation potential, proliferation, survival, or migration of the cells. In initial experiments, we showed that as few as 1,000 MSCs carrying iron oxide nanoparticles can be detected by MRI one month after their coinjection with breast cancer cells that formed subcutaneous tumors. Subsequently, we show that i.v.-injected iron-labeled MSCs could be tracked in vivo to multiple lung metastases using MRI, observations that were confirmed histologically. This is the first study to use MRI to track MSCs to lung metastases in vivo. This technique has the potential to show MSC integration into human tumors, allowing early-phase clinical studies examining MSC homing in patients with metastatic tumors. [Cancer Res 2009;69(23):8862–7]

Introduction

The poor survival of both lung cancer patients and those with other forms of pulmonary metastatic disease relates partly to the inability to deliver locally targeted therapeutic agents. A recent body of work has used exogenous mesenchymal stem cells (MSC) from the bone marrow compartment to attenuate several carcinoma models (1–6). In some of these studies, the MSCs carrying antitumor therapies have been delivered locally (2), whereas in others, they have been delivered systemically and migrate to the site of the tumor where they contribute to tumor reduction (1, 3–6).

We have shown previously in murine cancer studies that human MSCs expressing tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) can provide targeted delivery of this proapoptotic agent to breast cancer metastases (1). Similarly, MSCs transduced to express IFNβ or the immunostimulatory chemokine CX3CL1 have also been shown to reduce tumor burden in murine glioma (3), breast (5), melanoma (4), and colorectal models (6) with an improvement in survival.

The ability of bone marrow–derived stem cells to migrate to areas of injury in a range of pathologic conditions suggests that they may be ideal vectors for therapeutic delivery. MSCs possess a number of properties that make them suitable candidates. They are easily obtained from a simple bone marrow aspirate and are readily expanded in culture without losing their multilineage potential. They are readily transducible, allowing for simple ex vivo modification (7). Finally, they seem to be relatively nonimmunogenic (8) due to their lack of MHc2 and costimulatory molecules CD80, CD86, and CD40 (9). This may allow the delivery of genetically dissimilar MSCs without the need for immunomodulation or subsequent immunosuppressive therapy for the recipient. Because of these properties, MSCs have considerable therapeutic potential in tumor therapy.

To deliver cell therapy for cancer in the clinical setting, there is a need for imaging confirmation of targeted delivery. Novel imaging contrast agents have emerged that open up the possibility of visualizing stem cell transplants in vivo using magnetic resonance imaging (MRI). Superparamagnetic iron oxide (SPIO; Fe3O4) nanoparticles have been used for tracking engrafted cells in a variety of tissues (10), as well as targeted cell delivery (11). The nanoparticles generate a local magnetic field perturbation, which leads to a marked shortening of the MRI parameter T2*. This is exhibited as hypointensity on magnetic resonance images, leading to the possibility of imaging the localization of these particles (10, 12). We have exploited this phenomenon using MRI to track the fate of MSCs labeled with magnetic nanoparticles in a metastatic lung cancer model.

Here we have introduced biocompatible iron oxide nanoparticles into MSCs to enable localized cellular-level sensing while retaining full viability. Using a combination of cancer cells and iron nanoparticle–containing MSCs, we have shown in a subcutaneous tumor model that the MSCs can be imaged down to very low numbers. Finally, we show in a metastatic cancer model that systemically delivered cells can be tracked to pulmonary metastases, which is subsequently confirmed histopathologically.

Materials and Methods

Cell culture. Tissue culture reagents were purchased from Invitrogen unless otherwise stated. MDAMB231 breast cancer cells were obtained from Cancer Research UK, London Research Institute and were cultured in DMEM and 10% fetal bovine serum (FBS). Human adult MSCs were

Note: M.R. Loebinger and P.G. Kyrtatos are joint first authors. M.F. Lythgoe and S.M. Janes are joint last authors.

Requests for reprints: Mark F. Lythgoe, Centre for Advanced Biomedical Imaging, Department of Medicine and Institute of Child Health, University College London, Paul O’Gorman Building, 72 Huntley Street, London WC1 6BT, United Kingdom. Phone: 44-20-76796084; Fax: 44-20-76796073; E-mail: m.lythgoe@ich.ucl.ac.uk or Sam M. Janes, University College London, Rayne Building, 5 University Street, London WC1E 6JF, United Kingdom. Phone: 44-20-73690064; Fax: 44-20-73690067; E-mail: s.janes@ucl.ac.uk. ©2009 American Association for Cancer Research. doi:10.1158/0008-5472.CAN-09-1912

Published Online First on November 17, 2009 as 10.1158/0008-5472.CAN-09-1912

Published OnlineFirst November 17, 2009; DOI: 10.1158/0008-5472.CAN-09-1912
MRI of MSCs Homing to Metastases with Iron Nanoparticles

A. Intravital microscopy images showing cytoplasmic location of SPIO nanoparticles (yellow), DiI (red), and the nuclear counterstain DAPI (blue). B. The SPIO-loaded MSCs proliferate at the same rate as nonlabeled MSCs. C, D, the SPIO-loaded MSCs migrate through the transwell membrane at the same rate as nonlabeled MSCs (Non-Fe).

In vitro use of iron-labeled MSCs. In the subcutaneous model, varying numbers of MSCs labeled with CM-DiI (Invitrogen, as per manufacturer’s instructions) and iron nanoparticles were delivered concurrently with the cancer cells. In metastatic models, 7.5 × 10^5 MSCs were suspended in 200-μL PBS and injected into the lateral tail vein at day 35 after the metastatic model had been initiated (n = 4 mice). They were acquired using a fast spin-echo sequence with cardiac and respiratory gating [repetition time (TR) = 1 s, effective echo time (TE) = 5 ms, 100-μm in-plane resolution, 1-mm slice thickness, number of signals averaged (NSA) = 4]. Subcutaneous tumor images were obtained 28 d after s.c. injection of MDAMB231 cells and MSCs, and were acquired ex vivo using the same sequence and similar parameters (TR = 1.5 s, effective TE = 5 ms, 100-μm in-plane resolution, 1-mm slice thickness, NSA = 4; n = 14 mice; 2 per group). Signal-to-noise ratios (SNR) were obtained from three consecutive coronal slices for four lung areas (right and left, upper and lower).

Purchased from Tulane University (New Orleans, LA) and cultured in αMEM with 16% FBS. Both cell lines were obtained directly from cell banks that perform cell line characterizations [DNA fingerprinting and short tandem repeats (MDAMB231)] and were passaged for less than 6 mo. FluidMAG iron nanoparticles (NC-D, Chemicell GmbH) with a hydrodynamic diameter of 200 nm and a magnetite core were coated by the manufacturer with starch.

Labeling, phenotyping, and visualization of MSCs with iron nanoparticles. Labeling of MSCs with iron nanoparticles was done by overnight incubation with 0.5 mg/mL of nanoparticles in cell culture medium. The cells were vigorously washed with PBS eight times to remove any free particles before use.

Adipogenic and osteogenic differentiation of MSCs was done as previously described (13, 14). Cell viability was done using an MTS NAD(P)H-dependent assay (15) according to the manufacturer’s guidelines (Promega). Cell apoptosis was analyzed using an Annexin V-FITC/propidium iodide (PI) assay (ApoTarget, Invitrogen) 72 h after labeling. Ten samples were analyzed using a flow cytometer (FACSCalibur), and 6 × 10^3 to 8 × 10^3 cells were scored per analysis (CellQuestPro, Becton Dickinson). Annexin V−/PI− cells were kept in filter cages. Annexin V+/PI− cells were considered to be undergoing apoptosis, and Annexin V+/PI+ cells were considered late apoptotic or necrotic and recorded as dead (1).

Cell migration was done as previously described (1). Briefly, 1.5 × 10^5 MDAMB231 cells were plated in 800-μL medium on the bottom well of a transwell plate (Becton Dickinson), with 4 × 10^4 MSCs in 300-μL plated in the upper well. The MSCs were allowed to migrate across the 8-μm pore membrane for 24 h at 37°C. The cells that attached to the upper side of the membrane were removed with a cotton bud, and the cells on the lower side that had migrated through the membrane were fixed, stained (Rapid Romanowsky, Raymond Lamb), and counted (five fields per well, triplicate wells) at ×10 magnification (Olympus BX40).

Prussian blue staining (1.2% potassium ferrocyanide with 1.8% hydrochloric acid) was done on fixed cells (4% paraformaldehyde) 96 h after labeling. Confocal microscopy was done on a Leica TCS SP2 microscope (Leica Microsystems Ltd.). Reflectance was used to visualize iron as previously described (12), and images were processed using Image J. For electron microscopy, cells were fixed with 2% paraformaldehyde, 1.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3). They were then osmicated in 1% OsO4/0.1 mol/L phosphate buffer, dehydrated in a graded ethanol-water series, cleared in propylene oxide, and infiltrated with Araldite resin. Ultrathin sections were cut using a diamond knife, collected on 300 mesh grids, and then stained with uranyl acetate and lead citrate. These were viewed with a Jeol 1010 transmission electron microscope (Jeol) and the images were recorded using a Gatan Orius CDD camera (Gatan).

Iron quantification. We used a superconducting quantum interference device (16) to measure the amount of Fe3O4 in the cells. The samples were saturated in a field of 2 T, which was subsequently removed to leave the SPIO particles in a magnetized state. Comparison of this remnant signal saturated in a field of 2 T, which was subsequently removed to leave the SPIO nanoparticles in an ex vivo state [by comparison to a control signal acquired using the same sequence and similar parameters (TR = 1.5 s, effective TE = 5 ms, 100-μm in-plane resolution, 1-mm slice thickness, NSA = 4)].

Xenograft cancer models. All animal studies were done in accordance with the British Home Office procedural and ethical guidelines. Six-week-old nonobese diabetic/severe combined immunodeficient mice (Harlan) were kept in filter cages. Subcutaneous tumors were produced by the injection of 2 × 10^6 MDAMB231 cells in 200-μL PBS, s.c., into the left flank with a 29-gauge needle (17). Metastatic lung tumors were produced by the delivery of 2 × 10^6 MDAMB231 cells in 200-μL PBS into the lateral tail vein (1).

In vivo use of iron-labeled MSCs. In the subcutaneous model, varying numbers of MSCs labeled with CM-DiI (Invitrogen, as per manufacturer’s instructions) and iron nanoparticles were delivered concurrently with the cancer cells. In metastatic models, 7.5 × 10^5 MSCs were suspended in 200-μL PBS and injected into the lateral tail vein at day 35 after the cancer cells had been set up. As controls, MSCs not bearing nanoparticles, 100 ng of free iron, or iron nanoparticle–labeled MSCs, which were killed in 70% ethanol (cell death confirmed with trypan blue staining), were delivered with the cancer cells.
The labeled cells retained their MSC characteristics, with the ability to differentiate into stromal tissues, including bone and fat (Fig. 1Ai–iv). Furthermore, the iron nanoparticle–labeled and unlabeled MSCs showed equivalent in vitro tumor homing (104.4 ± 5.6 versus 113.1 ± 16.1 cells per field) in transwell migration studies [nonsignificant (ns), t test; Fig. 1B]. There was also no effect of iron nanoparticles on MSC proliferation (ns, two-way ANOVA), as shown by the MTS proliferation assay (Fig. 1C), or on cell viability, as shown by Annexin V flow cytometry apoptosis assay [33.0 ± 4.2% dead cells or apoptotic cells with no iron nanoparticles, compared with 29.4 ± 2.1% cells with iron nanoparticles (ns, Mann-Whitney); Fig. 1D].

Detection and sensitivity of MRI to iron-labeled cells. To determine the sensitivity of MRI in visualizing MSCs carrying iron nanoparticles, we used subcutaneous tumors, rather than lung tissue, in combination with our lung imaging MRI sequence to assess the dose response of iron-labeled cells, as the air spaces in the lung could confound this assessment. We grew subcutaneous MDAMB231 tumors (2 × 106 cells) in nonobese diabetic/severe combined immunodeficient mice with increasing numbers of DiI-labeled human MSCs carrying nanoparticles (100, 1 × 103, 1 × 104, and 1 × 105) for 28 days (n = 2 per group). Using a 9.4-T MRI system, we were able to visualize as few as 1,000 MSCs labeled with nanoparticles in tumors 28 days after injection of the MDAMB231 cells (Fig. 2Ai–iii). Signal voids were not visible at 28 days when non–iron-labeled MSCs, dead iron-labeled MSCs (Fig. 2Av), or free iron (Fig. 2Aiv) was coadministered with the tumor cells. Histopathologic examination confirmed that iron was present only in the tumors injected with live nanoparticle-labeled MSCs. This was shown by colocalization of the Prussian blue staining of iron and Dil fluorescence with the MSCs (Fig. 2B).

**Results**

Iron labeling of MSCs. The MSCs readily internalized the iron nanoparticles. This was confirmed by Prussian blue staining, electron microscopy, and confocal microscopy (Fig. 1Ai–iv). Cells contained up to 30 pg of iron oxide per MSC, quantified using superconducting quantum interference device magnetometry. The labeled cells retained their MSC characteristics, with the ability to differentiate into stromal tissues, including bone and fat (Fig. 1Ai–iv). Furthermore, the iron nanoparticle–labeled and unlabeled MSCs showed equivalent in vitro tumor homing (104.4 ± 5.6 versus 113.1 ± 16.1 cells per field) in transwell migration studies [nonsignificant (ns), t test; Fig. 1B]. There was also no effect of iron nanoparticles on MSC proliferation (ns, two-way ANOVA), as shown by the MTS proliferation assay (Fig. 1C), or on cell viability, as shown by Annexin V flow cytometry apoptosis assay [33.0 ± 4.2% dead cells or apoptotic cells with no iron nanoparticles, compared with 29.4 ± 2.1% cells with iron nanoparticles (ns, Mann-Whitney); Fig. 1D].

Detection and sensitivity of MRI to iron-labeled cells. To determine the sensitivity of MRI in visualizing MSCs carrying iron nanoparticles, we used subcutaneous tumors, rather than lung tissue, in combination with our lung imaging MRI sequence to assess the dose response of iron-labeled cells, as the air spaces in the lung could confound this assessment. We grew subcutaneous MDAMB231 tumors (2 × 106 cells) in nonobese diabetic/severe combined immunodeficient mice with increasing numbers of DiI-labeled human MSCs carrying nanoparticles (100, 1 × 103, 1 × 104, and 1 × 105) for 28 days (n = 2 per group). Using a 9.4-T MRI system, we were able to visualize as few as 1,000 MSCs labeled with nanoparticles in tumors 28 days after injection of the MDAMB231 cells (Fig. 2Ai–iii). Signal voids were not visible at 28 days when non–iron-labeled MSCs, dead iron-labeled MSCs (Fig. 2Av), or free iron (Fig. 2Aiv) was coadministered with the tumor cells. Histopathologic examination confirmed that iron was present only in the tumors injected with live nanoparticle-labeled MSCs. This was shown by colocalization of the Prussian blue staining of iron and Dil fluorescence with the MSCs (Fig. 2B).

**Results**

Iron labeling of MSCs. The MSCs readily internalized the iron nanoparticles. This was confirmed by Prussian blue staining, electron microscopy, and confocal microscopy (Fig. 1Ai–iv). Cells contained up to 30 pg of iron oxide per MSC, quantified using superconducting quantum interference device magnetometry. The labeled cells retained their MSC characteristics, with the ability to differentiate into stromal tissues, including bone and fat (Fig. 1Ai–iv). Furthermore, the iron nanoparticle–labeled and unlabeled MSCs showed equivalent in vitro tumor homing (104.4 ± 5.6 versus 113.1 ± 16.1 cells per field) in transwell migration studies [nonsignificant (ns), t test; Fig. 1B]. There was also no effect of iron nanoparticles on MSC proliferation (ns, two-way ANOVA), as shown by the MTS proliferation assay (Fig. 1C), or on cell viability, as shown by Annexin V flow cytometry apoptosis assay [33.0 ± 4.2% dead cells or apoptotic cells with no iron nanoparticles, compared with 29.4 ± 2.1% cells with iron nanoparticles (ns, Mann-Whitney); Fig. 1D].

Detection and sensitivity of MRI to iron-labeled cells. To determine the sensitivity of MRI in visualizing MSCs carrying iron nanoparticles, we used subcutaneous tumors, rather than lung tissue, in combination with our lung imaging MRI sequence to assess the dose response of iron-labeled cells, as the air spaces in the lung could confound this assessment. We grew subcutaneous MDAMB231 tumors (2 × 106 cells) in nonobese diabetic/severe combined immunodeficient mice with increasing numbers of DiI-labeled human MSCs carrying nanoparticles (100, 1 × 103, 1 × 104, and 1 × 105) for 28 days (n = 2 per group). Using a 9.4-T MRI system, we were able to visualize as few as 1,000 MSCs labeled with nanoparticles in tumors 28 days after injection of the MDAMB231 cells (Fig. 2Ai–iii). Signal voids were not visible at 28 days when non–iron-labeled MSCs, dead iron-labeled MSCs (Fig. 2Av), or free iron (Fig. 2Aiv) was coadministered with the tumor cells. Histopathologic examination confirmed that iron was present only in the tumors injected with live nanoparticle-labeled MSCs. This was shown by colocalization of the Prussian blue staining of iron and Dil fluorescence with the MSCs (Fig. 2B).
Homing and in vivo detection of iron-labeled MSCs to lung tumors. In the following experiments, $2 \times 10^6$ MDAMB231 cells were injected into the tail vein. This model reproducibly forms pulmonary metastases throughout all lung lobes. We were able to detect lung metastases using MRI, visualized as focal regions of increased signal. These areas correspond to metastases on H&E histologic sections (bar, 100 μm). One hour after SPIO-loaded MSC injection, there is a decrease in signal intensity caused by the iron oxide in MSCs (+, ribcage; *, trachea; ^, diaphragm with upper abdomen below; ∼, fissure separating lobes). B, the reduction in signal intensity secondary to SPIO-loaded MSCs 1 and 24 h after MSC injection was further confirmed and quantified by comparing SNR between the lung parenchyma and the deltoid muscle in three consecutive MR slices in three mice; there was a significant ($P = 0.005$) reduction in SNR across all four radiological areas (left upper (LU), left lower (LL), right upper (RU), and right lower (RL)). C, tumor histology from mice harvested at day 35, 1 h after SPIO-loaded MSC injection and MRI. Prussian blue (i) and DiI staining (ii; red) on contiguous sections from mice, showing that MSCs migrate to and incorporate into lung metastases after i.v. delivery (bar, 20 μm). iii, macrophage immunohistochemistry (brown) stains different cells from SPIO-loaded cells (blue stain). iv, macrophage immunofluorescence (green) stains different cells from DiI-labeled (red) cells (bar, 5 μm).

Discussion

MSCs have enormous potential as vehicles for directed cancer delivery. The mechanism responsible for the homing of MSCs to tumors is thought to involve chemokine ligands and receptors as with the recruitment of leukocytes to areas of inflammation. However, unlike with leukocytes, the specific chemokines responsible...
for MSC migration are less well characterized (1, 20). Nevertheless, homing to tumors has been confirmed by many studies using the labeling of MSCs and subsequent immunohistochrometry (21). Identification of the MSCs in these previous studies has necessitated histologic tissue and animal sacrifice. In this preliminary study, we have shown the ability to detect and visualize homing of iron-labeled MSCs in real-time in vivo.

For clinical applications, the ability to track MSC homing to primary tumors and metastases using a simple noninvasive scan would be of great benefit. Although murine models have shown a lot of promise for transduced MSCs in cancer therapy, many uncertainties still remain. The ability to systematically visualize the therapy and the response of the tumor will allow for more informed decisions about the optimum timing of MSC therapy, as well as the number of treatments.

We have previously described the use of MSCs in delivering the proapoptotic protein TRAIL (1). In these experiments, the expression of TRAIL was sensitively controlled by doxycycline via an inducible lentivirus. The ability to detect the proximity of the transduced MSCs to the tumors with MRI could be used in such a model, expressing the antitumor agent only when the MSCs are in their optimal position. Although the spatial proximity of the MSC homing to the tumors and the benefit of magnetic resonance monitoring for TRAIL therapy were not investigated as part of this study, we envisage that MSC tracking will help define the optimal time window by detection of MSCs in the lung and any regression of the metastases.

The noninvasive tracking of MSCs has previously been studied with the use of bioluminescence (22) and whole-body micro-potential conflict of interest were disclosed.

Acknowledgments

Received 5/26/09; revised 9/10/09; accepted 9/29/09; published OnlineFirst 11/17/09.

Grant support: M.R. Loebinger is a Medical Research Council (MRC) Clinical Training Fellow, S.M. Janes was an MRC Clinician Scientist. This work was partly undertaken at UCLH/UCL, which received a proportion of funding from the Department of Health NIHR Biomedical Research Centres funding scheme (S.M. Janes), the Institute of Child Health, Child Health Research Appeal Trust, the A.S. Onassis Public Benefit Foundation (P.G. Kyrtatos), the Biotechnology and Biological Sciences Research Council (A.N. Price and M.F. Lythgoe), the British Heart Foundation (M.F. Lythgoe), an Engineering and Physical Sciences Research Council Nanotechnology Grand Challenge Grant (Q. Pankhurst), and King’s College London and UCL Comprehensive Cancer Imaging Centre Cancer Research UK and Engineering and Physical Sciences Research Council, in association with the MRC and DoH, England (M.F. Lythgoe).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


Cancer Res 2009; 69: (23), December 1, 2009 8866 www.aacrjournals.org

References

Magnetic Resonance Imaging of Mesenchymal Stem Cells Homing to Pulmonary Metastases Using Biocompatible Magnetic Nanoparticles

Michael R. Loebinger, Panagiotis G. Kyrtatos, Mark Turmaine, et al.

Cancer Res  Published OnlineFirst November 17, 2009.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-09-1912