International Journal on Magnetic Particle Imaging
Vol 8, No 1, Suppl 1, Article ID 2203003, 3 Pages

Infinite Science Publishing

Proceedings Article

MPI of SuperSPIO20-labeled ALS patient-derived, genome-edited iPSCs and iPSC-derived motor neurons

A. Shakeri-Zadeh[a,b] · M. O'Brien[c] · A. Johns[c] · B. Tiret[d] · A. Bibic[a,e] · N. J. Maragakis[c] · G. Cotin[f] · B. Ayela[f] · D. Felder-Flesch[f] · J. W. M. Bulte[a,*]

[a] The Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, Baltimore, MD, USA
[b] Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, Baltimore, MD, USA
[c] Department of Neurology, the Johns Hopkins University School of Medicine, Baltimore, MD, USA.
[d] Magnetic Insight Inc., Alameda, CA, USA.
[e] F.M. Kirby Research Center for Functional Brain Imaging, Hugo W. Moser Institute, Kennedy Krieger , Baltimore, MD, USA
[f] SuperBranche, Strasbourg, France
[*] Corresponding author, email: jwmbulte@mri.jhu.edu

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Abstract

Genome-edited induced pluripotent stem cells (iPSCs), iPSC-derived neural precursor cells (NPCs) and iPSC-derived motor neurons (MNs) have shown considerable potential for neurorepair in transgenic amyotrophic lateral sclerosis (ALS) rodent models. When pursuing mutant gene-edited iPSC cell therapy in patients, it is highly desirable to have non-invasive imaging techniques available that can report longitudinally on the fate of transplanted cells. With magnetic particle imaging (MPI), one can visualize and quantify the distribution of superparamagnetic iron oxide (SPIO)-labeled stem cells in the body. Here, we report an optimized magnetic labeling protocol for MPI tracking of gene-edited iPSCs and iPSC-derived MNs. We used SuperSPIO20® and Resovist® for cell labeling and found that the MPI performance of SuperSPIO20® is about 20% higher than that for Resovist® when it comes to imaging of labeled cells. Furthermore, we compared the detection sensitivity of MPI with T2-W MRI and concluded that MPI has at least 10-fold higher sensitivity in cell detection.

I. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by rapid and unremitting degeneration of both upper and lower motor neurons (MNs). A mutation in the gene superoxide dismutase 1 (SOD1) is the main causative factor for developing familial ALS, occurring in approximately 20% of patients. Induced pluripotent stem cell (iPSC)-based therapies aimed at restoring the presence of normal MNs in patients with ALS represents an attractive therapeutic approach, but patient-derived cells cannot be used until genetically corrected (1). The process of deriving matured MNs from genome-edited iPSCs, mediated by neural precursos cells (NPCs), is shown in Figure 1. When pursuing mutant SOD1-edited iPSC cell therapy in patients, it is highly desirable to have non-invasive imaging techniques available that can report longitudinally on the fate

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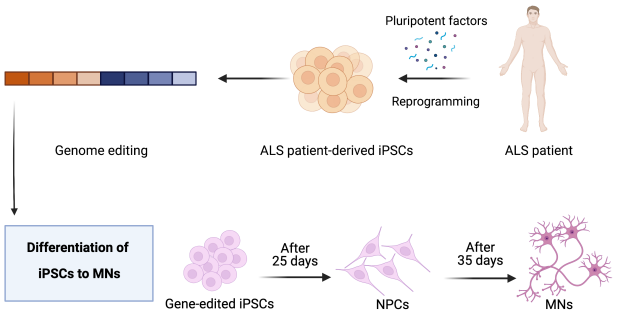

Figure 1: Differentiation of patient-derived genome-edited iPSCs to MNs, as a potentially valuable resource for cell replacement in ALS.

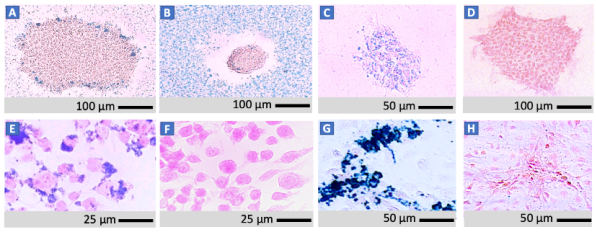

Figure 2: Prussian blue staining of magnetic labeling of iPSCs, NPCs, and MNs with SuperSPIO20. Panels A and B show the non-desirable, non-specific binding when using conventional matrigel coating. Panel C shows proper labeling of iPSCs using our modified protocol. In panel D, unlabeled iPSCs are shown for comparison. Bottom row shows (E) labeled NPCs, (F) unlabeled NPCs, (G) labeled MNs, and (H) labeled MNs.

of transplanted cells. Magnetic particle imaging (MPI) is a cellular and molecular imaging modality with future potential for clinical applications (2). With MPI, one can visualize and quantify the distribution of biocompatible superparamagnetic iron oxide (SPIO) nanoparticle tracers and SPIO-labeled stem cells in a specific manner (3). To track mutant SOD1-edited iPSCs in vivo with MPI, as part of the NIH somatic cell gene editing program (4), we developed an optimized magnetic labeling protocol for this purpose. We also compared the sensitivity of MPI with T2-weighted (W) MRI for detection of labeled cells.

II. Material and methods

The 39B2.5 iPSC cell line was used as an ALS patient-derived mutant SOD1-edited iPSC model and then differentiated into MNs through NPCs as intermediate cell type (1). Undifferentiated and differentiated cells were magnetically labeled with and without poly-L-lysine (PLL) as transfection agent and the commercial SPIO formulations Resovist® and dendronized SuperSPIO20® (5). Matrigel and laminin-coating of tissue culture plates, needed for structural support of iPSCs and MNs, was

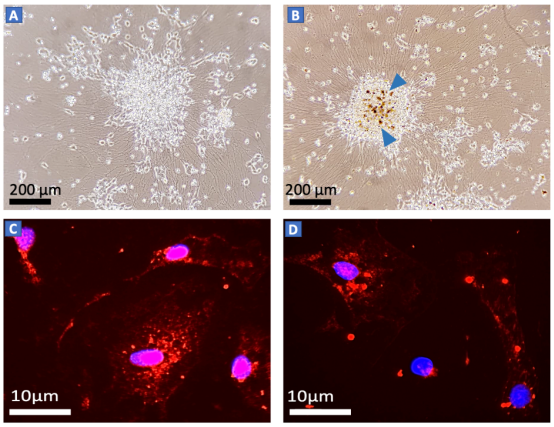

Figure 3: Bright field images of live (A) unlabeled and (B) SuperSPIO20-labeled NPCs at 39 days during the process of differentiation into MNs. Panel B shows a live cluster of labeled NPCs two weeks after labeling, with brown spots representing SuperSPIO20 nanoparticles (arrow heads). Anti-ChAT immunostaining (red) of NPC-derived cells shows proper differentiation into MNs for both (C) unlabeled and (D) SuperSPIO-labeled NPCs.

problematic for proper labeling as the coating absorbed near all SPIOs added to the medium (Figure 2). To overcome this obstacle, cells were first collected and then incubated with 75 µg Fe (with or without 1125 ng PLL) per ml of medium in 6-well ultra-low attachment plates for six hours. Prussian Blue staining and a Ferrozine-based spectrophotometric assay were used to assess intracellular iron uptake. MRI was performed at 11.7T using a horizontal bore Bruker Biospec scanner. T2-W images were acquired using a TurboRARE sequence with TR=2500 ms, effective TE=28 ms, NEX=2; TA=160 s, RARE factor=8; number of slices=9, slice thickness=1 mm, matrix size=256x256, and FOV=35x35 mm. MPI was performed with standard mode using a Magnetic Insight Momentum scanner using a linear holder. Two dimensional images were acquired with gradient strength=5.7 T/m, RF amplitude=20 mT in X and Z channels, and FOV= 6x12 cm per projection.

III. Results and discussion

Figures 2 A and B show a main limitation of SPIO-labeling of iPSCs using conventional matrigel-coated tissue culture plates, where the particles are bound non-specifically to the coated plate surface. Figures 2 C and D show properly labeled and unlabeled iPSC colonies using our revised, optimized labeling protocol. Results for labeled and unlabeled NPCs and MNs are shown in Figure 2 E-H.

The viability of SuperSPIO20-labeled iPSCs, iPSC-derived NPCs, and MNs was 94-99%. The percentage of MN-positive cells (choline acetyl transferase or ChAT-

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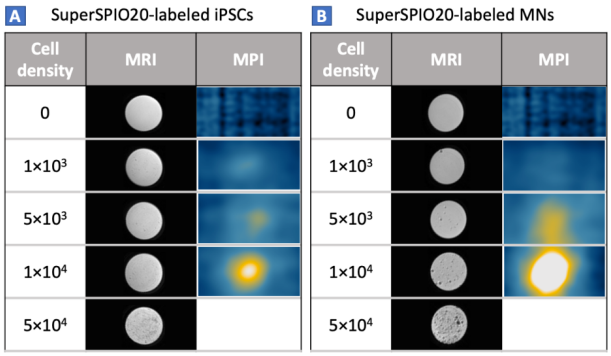

Figure 4: Comparison of MRI and MPI detection sensitivity for SuperSPIO20-labeled (A) iPSCs and (B) MNs. MPI has a 10-fold higher detection sensitivity.

positive cells) was similar for SuperSPIO20-labeled and unlabeled NPCs (Figure 3).

SuperSPIO20 and Resovist labeled cells equally, but only when PLL was used for Resovist, whereas for SuperSPIO20 this was not required. The MPI performance of SuperSPIO20 was about 20% higher than that for Resovist. The lower limit of detection for MPI and MRI using SuperSPIO20 was $5x10^3$ and $5x10^4$ cells/500 µl, respectively (Figure 4), with iron uptake levels between 9-13 pg Fe/cell.

IV. Conclusions

Optimizing protocols is imperative for each specific cell type. We presented here an optimized protocol for magnetic labeling of SOD1-edited iPSCs and their NPC and MN progeny. Using the same labeling protocols, MPI has a >10-fold higher detection sensitivity for iPSCs and iPSC-derived MNs compared to MRI. With our newly installed Momentum MPI scanner, we are now performing in vivo tracking studies of genome-edited iPSC-derived cells in a transgenic mouse model of ALS.

Acknowledgments

The authors acknowledge Dr. Kevin C. Eggan for providing SOD1-edited iPSCs. Research funding: This research was funded by grants from the National Institutes of Health (UH2/UH3 EB028904 and S10 OD026740) and the Maryland Stem Cell Research Fund (MSCRFD-5416).

Author's statement

Conflict of interest: Dr. Bulte receives grant support from Philips Healthcare, Inc., and is a paid consultant to SuperBranche. These arrangements have been reviewed and approved by the Johns Hopkins University in accordance with its conflict-of-interest policies. Drs. BA, GC, and DFF are employees of SuperBranche. Other authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: This research was performed in accordance with JHU institutional review guidelines.

References

[1] E. Kiskinis, et al., Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant SOD1, Cell Stem Cell. 2014;14(6):781-95.

[2] J.W.M. Bulte, Superparamagnetic iron oxides as MPI tracers: A primer and review of early applications. Advanced Drug Delivery Reviews. 2019; 138:293-301.

[3] J.W.M. Bulte, et al., Quantitative "hot-spot" imaging of transplanted stem cells using superparamagnetic tracers and magnetic particle imaging. Tomography. 2015;1(2):91-98.

[4] K. Saha, et al., The NIH somatic cell genome editing program. Nature. 2021;592(7853):195-204.

[5] G. Cotin, et al., Dendron based antifouling, MRI and magnetic hyperthermia properties of different shaped iron oxide nanoparticles. Nanotechnology. 2019;30(37):374002-19.

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