Journal of Nuclear Medicine, published on May 19, 2016 as doi:10.2967/jnumed.116.173963

Imaging the biodistribution and performance of transplanted stem cells with PET

Christopher G. England,[1] Emily B. Ehlerding,[1] and Weibo Cai[1,2]

[1] Department of Medical Physics, University of Wisconsin – Madison, Madison, Wisconsin, USA

[2] Department of Radiology, University of Wisconsin – Madison, Madison, WI, USA

[3] University of Wisconsin Carbone Cancer Center, Madison, Wisconsin, USA

First Author: Christopher G. England, University of Wisconsin – Madison, Rm. 7148, 1111 Highland Ave. Madison, WI 53705, USA. Email: cengland2@wisc.edu

Corresponding Author: Weibo Cai, University of Wisconsin – Madison, Rm. 7137, 1111 Highland Ave., Madison, WI 53705, USA. Email: wcai@uwhealth.org

Keywords: Positron emission tomography (PET); ^{18}F-Fallypride; human dopamine D2 receptor; muscle precursor cells; cell tracking; cell-based therapies; muscle regeneration

Several clinical trials have assessed the efficacy of transplanted stem cells for treatment of muscle-related diseases (*1*), yet clinical translation of these cell-based therapies has been limited by the host immune response, ineffective homing strategies for getting the donor cells to targeted musculature, and the intricate generation of large numbers of transplantable cells. Since highly invasive tissue biopsies are often required for diagnosing and monitoring the therapeutic intervention of muscle-related diseases, there is a dire need for alternative non-invasive approaches in the medical community. Recently, positron emission tomography (PET) imaging has shown great potential for tracking disease progression and monitoring therapeutic intervention through the use of biomarkers. More specifically, cell tracking with PET imaging can be accomplished by directly labeling cells of interest with an imaging tag or by indirectly labeling the cells with a reporter. This allows for scientists to track the fate of injected cells in vivo, virtually eliminating the need for highly invasive procedures in addition to providing fast and reliable data.

In this issue of *The Journal of Nuclear Medicine*, Haralampieva et al. from ETH Zurich reported on the non-invasive PET imaging of hMPCs in a nude mouse model using a signaling-deficient human dopamine D2 receptor (hD2R) as a reporter for binding of ^{18}F-Fallypride (*2*). There were three distinct reasons why the authors chose hD2R as the reporter gene. First, hD2R has been extensively studied, and expression of this dopaminergic receptor is normally restricted to the striata nigra brain region. This simplified the tracking of transplanted cells expressing hD2R in peripheral body regions as these tissues do not natively express hD2R. Second, hD2R may be genetically

mutated to eliminate the potential activation of signaling transduction pathways upon binding of hD2R ligands. Normally, binding of hD2R by its native ligands results in activation of a G-protein signaling pathway; thus, the activity of this receptor needed to be silenced to ensure that the genetically-altered hMPCs displayed the same phenotypic properties as normal hMPCs (*3*). By genetically uncoupling the binding activity from the intracellular signaling of hD2R, the activity of hPMCs would be unaffected by binding of an endogenous ligand or targeted imaging agent. Lastly, there are several highly efficient PET imaging agents already available for imaging hD2R. Next, the authors chose a transfection method for inserting this mutated gene into the hMPCs.

An adenoviral construct was used to transfect cells with the mutated hD2R gene. After transfection, hD2R-transfected cells showed no signs of toxicity and the proliferation rate of transfected hMPCs remained unchanged. Together, these data verified the simplicity and high efficiency of the adenoviral system for rapid transfection of the hD2R gene into hMPCs. Next, the binding affinity of hMPCs_hD2R for ^{18}F-Fallypride was assessed *in vitro*. Of note, ^{18}F-Fallypride is a high-affinity D2/D3 antagonist radioactive tracer used for PET imaging of extrastriatal dopamine receptors in low concentrations (*4*). Also, the pharmacokinetic properties and efficacy of ^{18}F-Fallypride have been extensively investigated, making it an optimal candidate for imaging of transplanted hMPCs_hD2R. Since the authors did not provide a rationale for choosing ^{18}F-Fallypride in this study, we predict that future studies may compare other commonly utilized hD2R-based PET tracers, including ^{124}I-Epidepride, ^{76}Br-Isoremoxipride, and perhaps their

^{11}C-labeled derivatives for tracking the biodistribution of hMPCs_hD2R transplanted *in vivo* (*5*).

To track the fate of hMPCs *in vivo*, nude mice were subcutaneously injected with transfected hMPCs_hD2R before PET imaging with ^{18}F-Fallypride. PET imaging revealed high uptake of ^{18}F-Fallypride in hMPC-hD2R at 1 week post-transplantation, whereas uptake of the tracer was significantly lower at 2 weeks post-transplantation. The authors attributed this signal decrease to the post-translational modification of ectopically expressed hD2R and/or the internalization and degradation of hD2R during myofibril differentiation. Low PET signal in the cerebellum brain region further confirmed the high specificity of ^{18}F-Fallypride for hMPCs_hD2R. In addition, PET studies were performed using haloperidol as a D2R blocking agent, in which hD2R-expressing cells and tissues showed decreased PET signal after the injection of haloperidol. Next, the authors investigated the oxygenation status of transplanted hMPCs_hD2R by PET imaging with ^{18}F-FMISO. Uptake of ^{18}F-FMISO was projected to be highest soon after cell transplantation, as low oxygen levels are found during the initial stages of cell-to-myofibril formation. Of note, ^{18}F-FMISO is the most widely investigated PET tracer for preclinical and clinical imaging of hypoxia. As expected, uptake of ^{18}F-FMISO was relatively high at 1 week post-transplantation, which was further corroborated by increased protein levels of the endothelial cell marker vWR and elevated vascular endothelial growth factor-A (VEGF-A) gene expression at later time points. Together, increased VEGF-A and endothelial cell markers, in combination with high oxygen consumption, are indicative of actively growing tissues. The authors concluded that ^{18}F-

FMISO allowed for sensitive monitoring of hypoxia-related biological processes in bioengineered constructs.

While this study provided a methodology for tracking hMPCs *in vivo*, this technology can be further implemented to track the fate of other transplanted cells. For example, Schonitzer et al. reported a similar approach for non-invasively tracking human mesenchymal stem cells utilizing a different mutated form of hD2R (*6*). As only 25% of the cells showed successful transfection of hD2R in the hMPCs, other transfection strategies may provide better transfection rates. In addition, reporter genes can complicate PET quantification and *in vivo* kinetic modeling of imaging tracers. Effective tracer uptake is dependent upon the availability of targeted cells, the injected tracer dose, the regional blood flow and vascularity of the tissue, and the pharmacokinetics of tracer uptake (*7*). It was previously described that reporter genes encoding for intracellular enzymes or transporters show superiority over membrane receptors like hD2R as they allow the tracer to accumulate inside the cell, whereas binding to surface receptors may be weak and reversible (*8*). In addition to the dopamine model described in this study, the sodium-iodide symporter transfection model is an alternative model which has been commonly employed for PET or SPECT imaging (*9*).

The clinical applicability of stem cell-based therapies is slowly becoming a potential reality. The utilization of cell-based therapies for the treatment of muscle-related diseases is promising, yet many uncertainties remain. First, transfected stem cells have been widely explored in basic preclinical research, yet these transfection models have

been limited to cell imaging and not therapeutic intervention. For this reason, clinical employment of reporter gene systems for patient treatment remains unlikely at this time. Currently, ^{18}F-FDG is the only PET tracer used in a clinical setting for stem cell tracking (*10*). Also, reporter gene models may be limited by changes in the expression of the transfected gene and encoded receptor. Additionally, the effects of the host immune system on the efficacy of cell-based therapies remain unclear. In the future, dual treatment strategies employing both gene and cell-based therapies may show additive or synergistic therapeutic effects in comparison to monotherapy-based approaches. As new cell-based therapeutic strategies are investigated, the non-invasive tracking of transplanted cells with high specificity using PET or other imaging modalities may eliminate the necessity for highly invasive procedures. As researchers continue to investigate novel cell-based therapies, molecular imaging must continue to evolve to support the need for non-invasive imaging approaches. This field holds great promise, and we expect that cell-based therapies will become a vital part of personalized medicine for the treatment of a broad range of diseases in the future.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This work was supported, in part, by the University of Wisconsin - Madison, the National Institutes of Health (NIBIB/NCI 1R01CA169365, P30CA014520, T32CA009206, and T32GM008505), and the American Cancer Society (125246-RSG-13-099-01-CCE).

REFERENCES

1. Skuk D. Myoblast transplantation for inherited myopathies: a clinical approach. *Expert Opin Biol Ther.* 2004;4:1871-1885.

2. Haralampieva D, Betzel T, Dinulovic I, et al. Non-invasive imaging and tracking of engineered human muscle precursor cells for skeletal muscle tissue engineering Using positron emission tomography. *J Nucl Med.* 2016. In Press.

3. Boyd KN, Mailman RB. Dopamine receptor signaling and current and future antipsychotic drugs. *Handb Exp Pharmacol.* 2012;212:53-86.

4. Vandehey NT, Moirano JM, Converse AK, et al. High-affinity dopamine D2/D3 PET radioligands 18F-fallypride and 11C-FLB457: a comparison of kinetics in extrastriatal regions using a multiple-injection protocol. *J Cereb Blood Flow Metab.* 2010;30:994-1007.

5. de Paulis T. The discovery of epidepride and its analogs as high-affinity radioligands for imaging extrastriatal dopamine D(2) receptors in human brain. *Curr Pharm Des.* 2003;9:673-696.

6. Schonitzer V, Haasters F, Kasbauer S, et al. In vivo mesenchymal stem cell tracking with PET using the dopamine type 2 receptor and 18F-fallypride. *J Nucl Med.* 2014;55:1342-1347.

7. Terrovitis JV, Smith RR, Marban E. Assessment and optimization of cell engraftment after transplantation into the heart. *Circ Res.* 2010;106:479-494.

8. Chen IY, Wu JC, Min JJ, et al. Micro-positron emission tomography imaging of cardiac gene expression in rats using bicistronic adenoviral vector-mediated gene delivery. *Circulation.* 2004;109:1415-1420.

9. Chung J-K. Sodium iodide symporter: Its role in nuclear medicine. *J Nucl Med*. 2002;43:1188-1200.

10. Kooreman NG, Ransohoff JD, Wu JC. Tracking gene and cell fate for therapeutic gain. *Nat Mater*. 2014;13:106-109.