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Molecular Imaging of Stem Cell Transplantation in Myocardial Disease

Jaehoon Chung and Phillip C. Yang

Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, 300 Pasteur drive, H-2157, Stanford, CA 94305, USA

Phillip C. Yang: pyang@cvmed.stanford.edu

Abstract

Stem cell therapy has been heralded as a novel therapeutic option for cardiovascular disease. In vivo molecular imaging has emerged as an indispensable tool in investigating stem cell biology post-transplantation into the myocardium and in evaluating the therapeutic efficacy. This review highlights the features of each molecular imaging modality and discusses how these modalities have been applied to evaluate stem cell therapy.

Keywords

Stem cells; Molecular imaging; Heart disease

Introduction

Stem cell therapy holds tremendous therapeutic potential for cardiovascular diseases. Several phase I clinical trials with bone marrow stem cell or endothelial progenitor cell transplantation have shown promising but equivocal results for ischemic heart disease [1–4]. One significant limitation in evaluating the data from these clinical trials is the lack of a reliable in vivo imaging method to assess the survival of the transplanted cells. Although echocardiography, radionuclide imaging, CT, or MRI has been used in these clinical trials to assess cardiac morphology, function, perfusion, and viability, no effort has been made to evaluate the biology of transplanted stem cells. Similarly, in large animal studies, the viability of transplanted stem cells is evaluated on postmortem histologic basis, which precludes longitudinal monitoring [5–8]. A fundamental but critical biological issue regarding the survival or engraftment of the transplanted cells could not be addressed fully. The transplanted stem cells must survive in the myocardium to generate beneficial therapeutic effects.

In vivo molecular imaging monitors and records the spatiotemporal distribution of molecular and cellular processes in an intact living organism. This innovative technique can be implemented to evaluate the seemingly complex underlying biology of cell therapy. Molecular imaging is well positioned to play a significant role to elucidate this cellular process by providing critical insights into post-transplantation stem cell survival, engraftment, and subsequent physiologic restoration of the recipient myocardium. Critical questions in stem cell therapy regarding optimal cell type, cell delivery efficiency, biodistribution, cell engraftment, and mechanism underlying myocardial restoration could be addressed by in vivo molecular

Correspondence to: Phillip C. Yang, pyang@cvmed.stanford.edu.

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imaging. This review addresses the current status of different molecular imaging modalities to evaluate stem cell therapy in cardiovascular disease.

Molecular Imaging Modalities

Echocardiography

Contrast agent technology incorporating microbubbles, liposomes, or nanoparticles has been developed to image stem cells [9]. These contrast agents can be conjugated with antibodies or ligands, leading to a specific accumulation of targeted contrast agents at the site of cell transplantation. Although most targeted ultrasound contrast agents are microbubbles, other vehicles can be used, including acoustically active liposomes and perfluorocarbon nanoparticles [10]. One of the earlier studies targeted the endothelial cells activated during an inflammatory response with lipid-shelled microbubble engineered with a monoclonal antibody to intercellular adhesion molecule (ICAM)-1 [11]. Subsequently, effective targeting of acoustically reflective liposomes to fibrin using anti-fibrinogen and anti-ICAM-1 was developed [12]. Recently, human CD133⁺ cells were labeled with CliniMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) nanoparticles, which tracked the delivery and fate of the cells in a porcine model of myocardial infarction using trans-esophageal echocardiography (Fig. 1) [13]. These advances, however, were limited by the acoustic window and the general constraints of ultrasound technology, resulting in limited spatial resolution, suboptimal image quality, and inadequate anatomical coverage. The exact relationship between the molecular signal of the transplanted cells and the surrounding myocardium could not be clearly delineated.

Radionuclide Imaging

Radionuclide imaging using single photon emission CT (SPECT) and positron emission tomography (PET) has been validated for evaluation of myocardial ischemia for decades. Recently, endothelial progenitor cells loaded with ¹¹¹In oxine were injected into the infarcted myocardium of nude rats. The SPECT images demonstrated that the radioactive signal generated from the transplanted cells in the myocardium was approximately twice the activity from the peripheral skeletal muscle tissue [14]. Furthermore, using PET imaging, bone marrow stem cells directly loaded intracellularly with ¹⁸F-fluorodeoxyglucose ([¹⁸F]-FDG) were monitored following injection into human subjects (Fig. 2) [15]. However, both of these studies were hampered by the short half-life of tracers, enabling limited capability to track the transplanted cells longitudinally. Moreover, detection of directly ex vivo-loaded stem cells with radionuclide would be problematic upon rapid proliferation or division of the transplanted cells, resulting in potential uptake of the radionuclide by bystander cells. Therefore, this imaging method would be able to localize the transplanted cells but would not be suitable to assess fundamental biology, such as the survival of the transplanted cells longitudinally.

Recent advances in reporter gene-based cell labeling have extended the capabilities of PET imaging to monitor stem cell viability longitudinally. The stem cells are typically transduced by a reporter gene ex vivo and transplanted into the target organ. Reporter genes, subsequently, encode for intracellular expression of enzymes or receptors, which is designed to interact with or bind to reporter gene-specific substrates or probes. Following transplantation, in vivo imaging with a PET-specific radionuclide-labeled reporter probe enables detection of the specific biology of the transplanted cells. Currently, the PET reporter gene employs the herpes simplex virus type 1 mutant thymidine kinase (*HSV1-tk*) system in conjunction with the [¹⁸F]-FDG radionuclide tracers. A triple fusion reporter gene for fluorescence, bioluminescence, and PET imaging has been developed to study embryonic stem cell survival, proliferation, and migration following delivery into the rat myocardium [16]. However, this approach requires ex vivo genetic manipulation of stem cells, and the long-term sustainability of the reporter gene expression has not been clarified.

Optical Imaging

In optical bioluminescence imaging, an ultrasensitive charge-coupled device camera captures the photon energy generated by the enzyme luciferase. The enzyme cleaves the luciferin substrate into metabolites that emit photons within a living cell without a need for an external light source [17]. This technique requires stable luciferase expression by stem cells and systemic injection of luciferin at each image acquisition. An optical imaging system is simpler, less expensive, and more convenient compared with other molecular imaging modalities. Robust viability signal has been detected as early as post-transplant day 3 in human embryonic stem cells transplanted into the peri-infarct area of the murine model of myocardial injury (Fig. 3) [18,19]. Although not precisely characterized to date, the sensitivity for detection is thought to be in the 10^{-15} to 10^{-17} mol/L range at limited depths of no more than 1 to 2 cm [20]. The tissue penetration of the emitted light strongly depends on both the thickness of the tissue and the type of tissue. These limitations preclude the use of this technique in larger animals.

MRI

MRI can provide high spatial and temporal resolution tomographic imaging with intrinsically superior soft tissue contrast. Consequently, cardiac MRI can provide accurate anatomical localization and assess the precise functional effect of stem cell therapy. MRI, however, suffers from a lower sensitivity in detecting the molecular signal from the transplanted stem cells in comparison with other imaging modalities. Yet, recent implementation of paramagnetic and superparamagnetic contrast agents in molecular MRI has shown successful generation of robust signal.

Superparamagnetic iron oxide nanoparticles (SPIOs) can induce a strong magnetic field inhomogeneity, consequently reducing T2* relaxation time and generating hypointense susceptibility artifacts. These properties enable robust visualization of SPIO-labeled stem cells through the strong hypointense signals described as a blooming effect [21]. Currently, two distinct classes of iron oxide nanoparticles are available in the United States based on the hydrodynamic particle size. The mean diameter of SPIO is approximately 100 to 500 nm. Ultrasmall superparamagnetic iron oxide particles are approximately 5 to 30 nm. Both nanoparticles have similar chemical structures. The core iron oxide nanoparticles are coated by dextran or other polymers to maintain solubility and reduce particle agglomeration. Because of this chemical structure, both of these agents are metabolized by the iron pathway and are biocompatible. Furthermore, cross-linked iron oxides have functionalized surfaces, which can conjugate targeted ligands, so the particles can be specifically linked to moieties such as vascular cell adhesion molecule-1 or annexin-5 [22,23]. SPIO is currently approved by the US Food and Drug Administration for imaging of liver lesions. Studies from our laboratory and others have shown that mouse and human embryonic stem cell viability and differentiation capacity is not altered with SPIO direct labeling [24]. However, one study reported in vitro differentiation capacity of mesenchymal stem cells into chondrocyte lineage was reduced after SPIO labeling in a dose-dependent manner, whereas osteogenic and adipogenic differentiation was intact [25]. Higher efficiency of iron-oxide labeling is achieved by adding transfection agents such as PLL, PS, or lipofectamine [24]. All these transfection agents neutralize the negatively charged SPIO to facilitate the attraction and binding of slightly positive or neutral complex to the negatively charged cell membrane. The mechanisms by which these complexes enter the cell have not been investigated conclusively but probably include a combination of endocytosis, invagination, or passive diffusion. In vivo experiments have demonstrated that SPIO-labeled cells provide high sensitivity to detect the anatomical location of the cells (Fig. 4) [26]. Despite increased sensitivity in the detection of the cells in the range of 10^{-9} mol/L reaching the sensitivity of radionuclide agents, this blooming effect produces a large signal void at the region of interest to confound the MRI signal from the surrounding artifact, corrupting the evaluation of the anatomy and physiology of the target tissue. In addition, ex

vivo SPIO direct labeling does not provide any biologic information such as the viability of transplanted stem cells because of the residual tissue deposition or nonspecific uptake by the macrophages of the SPIO particles in the surrounding tissue from dead or dividing SPIO-labeled stem cells [27,28].

Another cellular MRI contrast agent employs manganese (Mn^{2+}), an essential element, which can enter the cytoplasm of biologically active cells through the voltage-gated Ca^{2+} channels. These channels are known to have high affinity for Ca^{2+} analog, such as Mn^{2+} , which consequently accumulates within the intracellular cytoplasm through binding to specific sites on nucleic acid and intracellular proteins. Intracellular accumulation of Mn^{2+} induces a T1-shortening effect, which allows clear delineation of the cells of interest by generating hyperintense positive signal. Therefore, this contrast mechanism enables correlation between stem cell location and viability through a T1-weighted hyperintense signal [29,30]. Our studies have demonstrated that $MnCl_2$ direct labeling of human embryonic and mesenchymal stem cells can generate robust hyperintense signal without any significant alteration of viability or differentiation capacity. Furthermore, systemic administration of $MnCl_2$ has enabled reliable detection of human embryonic stem cell-derived teratoma formation in a severe combined immunodeficiency mouse model (Fig. 5) [30]. High concentration of Mn^{2+} has been reported to generate potential toxicity at the cellular level and create untoward systemic effects on the neurologic and cardiovascular functions. These untoward cardiovascular effects of $MnCl_2$, however, have been overcome by concurrent calcium supplementation [31].

In vivo molecular MRI employing reporter gene offers an alternative method to image and detect the specific biological processes of the transplanted stem cells. Several groups have shown successful in vivo applications of MRI reporter genes employing enzyme-, metalloprotein-, or receptor-based approaches for targeted molecular MRI [32–34]. So far, these techniques have not demonstrated successful tracking of transplanted stem cells in vivo. Currently, our group has demonstrated the feasibility of reporter gene-mediated in vivo molecular MRI of human embryonic stem cells. These stem cells have been bioengineered to express specific cell surface antigens if they are viable following transplantation into the murine myocardium. Systemic delivery of SPIO-conjugated monoclonal antibody specific to these surface antigens has demonstrated reliable in vivo tracking of the viable embryonic stem cells (Fig. 6) [35].

Conclusions

Recent clinical trials have begun to assess the potential efficacy of stem cell therapy in ischemic heart disease. Future trials establishing therapeutic benefits of this novel intervention are expected. However, the lack of a clear post-transplant mechanism underlying this benefit may hamper clinical acceptance of this novel therapy. In vivo molecular imaging will play a critical role in elucidating such a mechanism, including the fundamental biological evaluation of the survival of the transplanted stem cells. The cells must at the very least survive and engraft to restore the injured myocardium. There is no perfect single molecular imaging modality, and each technique has limitations. However, continued advances in each molecular imaging modality described here will facilitate the understanding of the biological mechanism and longitudinal monitoring of the efficacy of cell therapy.

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Fig. 1. Echocardiographic images of the posterolateral apex before (a) and after (b) engraftment of CliniMACs (Miltenyi Biotec, Bergisch Gladbach, Germany) magnetically labeled CD133⁺ cells. The magnetically labeled cells appeared hyperechoic (green arrows) in postdelivery imaging. (From Bara et al. [13]; with permission.)

Fig. 2. Myocardial homing and biodistribution of $[^{18}\text{F}]$ -FDG-labeled bone marrow cells. Unpurified bone marrow cells (**a** and **b**) show much lower homing to the infarcted myocardium than do CD34-enriched bone marrow cells (**c** and **d**). Nonmyocardial homing is also present in the liver and the spleen. (From Hofmann et al. [15]; with permission.)

Fig. 3.

In vivo bioluminescence imaging of severe combined immunodeficiency mice. Luciferase-transfected human embryonic stem cells were transplanted into murine ischemic-injured myocardium. The images were obtained by IVIS-spectrum (Caliper, Mountain View, CA) at post-transplant day 7. Significant luciferase activity is noted within the chest, suggesting in vivo cell proliferation

Fig. 4.

In vivo cardiac MRI of human embryonic stem cells (hESCs) transplanted into the murine myocardium. Following direct ex vivo labeling of hESCs with superparamagnetic iron oxide nanoparticles using protamine sulfate, 0.5×10^6 hESCs were transplanted into the left ventricle as indicated by negative dephasing signal (*black arrow*). The mouse was scanned by a 3 T clinical MRI scanner (GE Healthcare System, Milwaukee, WI)

Fig. 5.

In vivo manganese-enhanced MRI (MEMRI) of human embryonic stem cells (hESCs) transplanted into the murine hind limbs. Following transplantation of firefly luciferase-transfected hESCs into severe combined immunodeficiency mouse hind limb muscles, in vivo MEMRI was performed. The positive signal generated by hESCs following intraperitoneal administration of MnCl_2 is indicated by *yellow arrows*. The mouse was scanned using a 3 T clinical MRI scanner (GE Healthcare System). Concurrent bioluminescence imaging shows significant luciferase activity validating in vivo MEMRI signal detecting not only the location of the transplanted cells but also the viability of the cells

Fig. 6.

MRI reporter gene detection of the mouse embryonic stem cells (mESCs) transplanted into severe combined immunodeficiency (SCID) mouse myocardium. Following transplantation of MRI reporter gene-transduced mESCs into the SCID mouse myocardium, molecular MRI was acquired at post-transplantation days 7 and 14. Prior to each acquisition, superparamagnetic iron oxide nanoparticle-conjugated antibody specific to the cell surface antigens expressed by the MRI reporter gene was administered intravenously. Hypointense signal was generated by viable stem cells, indicated by *blue arrows*. Concurrent bioluminescence imaging shows positive luciferase activity validating MRI signal generated by the viable mESCs. Similarly, the hematoxylin and eosin staining validates MRI signal through correlation with the ex vivo mESCs location indicated by *red arrows*