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Bioimaging of Gene and Stem Cell Fate

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Abstract

Parallel advances in molecular imaging modalities and in gene- and cell-based therapeutics have significantly advanced their respective fields. Here we discuss how the collaborative, preclinical intersection of these technologies will facilitate more informed and effective clinical translation of relevant therapies.

In recent years, investigators have made impressive progress in the bench-to-bedside translation of gene- and stem cell-based therapies to address a wide range of pathologies in preclinical and clinical settings. Similar advances in bioimaging have provided powerful tools to monitor their *in vivo* fate and function. Here, we provide an overview of relevant gene- and cell-based therapy and highlight the applications of molecular imaging technologies to evaluate graft function, regulation, and interaction with host tissue. We emphasize particular strategies to facilitate the continued, collaborative synergy between molecular imaging technologies and gene- and stem cell-based therapeutics, which will expedite their assessment and development.

Clinical and preclinical experience

Corrective gene therapy in the clinics

Gene- and stem cell-based therapies hold potential to help treat a variety of diseases. Investigators have successfully illustrated the principle of isolating, engineering, and re-introducing a “corrected” graft for a variety of diseases with lineage-restricted phenotypes, including X-linked[1] and adenosine deaminase deficient[2] severe combined immunodeficiency disease, chronic granulomatous disease[3], adrenoleukodystrophy[4], and Wiskott-Aldrich Syndrome[5]. These therapies, largely limited experimentally to retroviral insertion of the corrected gene product in autologously derived hematopoietic stem cells (HSCs), have been met with widely publicized and appropriately directed concerns regarding their safety, despite encouraging demonstrations of phenotype correction. Follow-up reports have shown

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leukemic[6] and pre-leukemic[7] induction, clonal T cell expansion[8], and genomic instability[7] secondary to retroviral-mediated insertional mutagenesis in or near proto-oncogenes. Such events, due to untargeted genome editing, served as an impetus for the temporary Food and Drug Administration (FDA) ban on gene therapy in 2002. The subsequent lifting of the ban in 2003 heralded a more skeptical, and slow-progressing era that has continued to the present for a field yet to realize its full potential.

Allogeneic stem cell transplantation in the clinics

Though the aforementioned near pause in gene therapy led to more cautious development in this arena, interest in autologous or allogeneic stem cell-based approaches strengthened significantly. Despite wide interest in use of bone marrow-derived mesenchymal stem cells (MSCs) for a range of regenerative therapies, including those for inflammatory[9], joint[10], and cardiac diseases[11], among others, questions regarding the clinical efficacy of various stem cell protocols remain. In addition to marginal improvement observed in several stem cell trials, there is also evidence of detrimental side effects as seen with skeletal myoblast therapy for cardiac repair[12]. The discrepancy between the more definitive preclinical success of stem cell therapies and their less promising early clinical results may be partly attributed to a lack of knowledge regarding *in vivo* graft behavior.

Promising new therapeutic products are now emerging, in particular those making use of human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) derivatives. These include the now defunct Geron trial using allogeneic hESC-derived oligodendrocyte progenitor cells for spinal cord repair[13], the Advanced Cell Technology trial using hESC-derived retinal pigment epithelium cells (RPEs) for Stargardt's macular dystrophy[14], and the upcoming RIKEN Japan trial using autologous iPSC-derived RPEs for age-related macular degeneration[15]. As with earlier somatic cell therapies, pluripotent stem cell therapeutics will also need to be extensively tested and evaluated by bioimaging technologies to better understand their fate *in vivo*.

Genetically engineered stem cells

With the goal of optimizing clinical impact, the most promising approaches combine gene and cell therapy to deliver a corrected or beneficial gene in a therapeutically relevant cell. As an emerging paradigm, T cell immunotherapy offers hope for more targeted chemotherapy by genetically instructing T cell trafficking, direction, or redirection toward tumor cells, with the potential to engineer bi-specific T cells with engineered proliferation and anti-tumor specificities[16]. A noteworthy, single-patient case example of using PET imaging to track the fate of genetically-labeled and therapeutically-manipulated cytolytic T lymphocytes (CTLs) has demonstrated a valuable platform for integrating gene-cell therapy with molecular imaging[17]. In this report, CTLs labeled with the PET reporter gene herpes simplex virus thymidine kinase (*HSV-tk*) were infused intracranially after resection of a glioblastoma multiforme. PET imaging after administration of the PET reporter probe 9-(4-[18F]-fluoro-3-hydroxymethylbutyl)-guanine ([^{18}F]FHBG) revealed not only accumulation of the engineered CTLs in the patient's primary tumor but also homing to another metastatic site.

In addition to studying the *in vivo* fate of transplanted engineered cells, *ex vivo* edited cells also offer a valuable investigative platform. For instance, the ability to reprogram patient-specific adult somatic cells to iPSCs by overexpression of pluripotent transcription factors[18] has been used for *ex vivo* disease modeling. Notable examples of recapitulating disease phenotypes in a dish include amyotrophic lateral sclerosis[19], spinal muscular atrophy[20], long QT syndrome[21], and inherited cardiomyopathies[22,23], among others. Beyond disease modeling, this platform has also expedited development of high-throughput drug screening[24] as well as gene correction in monogenic diseases[25].

Bioimaging

From disease modeling to disease monitoring

Gene- and stem cell-based approaches have suffered from a lack of knowledge and control over *in vivo* graft behavior. Requiring years of preclinical testing, their combined progression will need to overcome the obstacles that have impeded these approaches independently and should benefit significantly from insights gained from bioimaging of gene and stem cell fate. Historically, lineage mapping by physical or genetic labeling has contributed extensively to our understanding of development and stem cell behavior, and assisted in the isolation of important cell populations. To better understand why gene and cell therapies have fallen short of their potential to date, an approach similar to that taken by developmental biologists should be more fully adopted by molecular imaging specialists and translational researchers. The coupling of therapeutic cells or vectors to reporter cassettes to permit live, longitudinal imaging of cellular processes may provide key insights that will help elucidate and harness their full regenerative and corrective capacities, while simultaneously addressing safety and regulatory concerns (Figure 1)[26,27].

Defining and labeling the therapeutic population

Imaging Modalities

For regenerative medicine, several cell types are of interest due to their multipotent (e.g., MSCs) or pluripotent (e.g., ESCs and iPSCs) nature. Therapeutic applications of some of these cells have been explored through clinical trials, but unresolved concerns surrounding safety and efficacy are limiting their full clinical implementation[28]. Chief barriers to full regulatory acceptance of pluripotent stem cell-based therapeutics are their immunogenic and tumorigenic potential; addressing these will require *in vivo* tracking of transplanted grafts[29]. *In vivo* tracking of cell fate involves either “direct” physical labeling of cells by incubating them with a contrast agent, or “indirect” genetic labeling of cells by transfecting them with reporter gene construct(s). The position of and signal from these labels can then be tracked using a charged coupled device (CCD) camera for bioluminescence imaging[30] or fluorescence imaging (FLI), single photon emission computed tomography (SPECT), positron emission tomography (PET), and magnetic resonance imaging (MRI), among other modalities. Selection of the optimal labeling technique and imaging modality depends on the cellular processes that need to be studied as well as the read-outs that are most desirable, with each labeling and imaging strategy having distinct advantages and disadvantages[31]. To date, most clinical studies have relied on “direct” labeling strategies to track homing and

migration of multipotent stem cells or engineered cells (see Table 1). These studies have answered critical questions in regenerative medicine, such as the importance of early stem cell engraftment on predicting late functional improvement[32] and the optimal route of cell delivery (comparing transendocardial versus intracoronary routes) into the heart[33]. In addition, these cardiac regenerative studies highlight the importance of combining bioimaging of organ function with that of cell homing to assess which part of a diseased organ might benefit most from cell therapy[34]. For immunotherapy using modified cytolytic T-lymphocytes or tumor-specific dendritic cells (DCs), bioimaging has provided clinicians with important insight into the kinetics of anti-tumor cell infiltration into tumor tissue[17,35]. By using fluorinated (^{19}F) contrast agents to label human DCs for MRI, pre-clinical studies have further demonstrated DC migration to draining lymph nodes, with superior assessment of cell quantity compared to that obtained by other MR imaging labels[36,37].

With the current focus on moving pluripotent stem cell derivatives to clinics[38], *in vivo* tracking of these cells is critical in assessing their homing and proliferative potential over time, as well as the exclusion of teratoma formation[39]. In preclinical studies, more emphasis is placed on “indirect” genetic labeling over “direct” physical labeling because the former technique is not subject to signal dilution upon cell division nor discordance between signal intensity and cell viability upon graft loss[40,41]. Of note, label uptake by inflammatory cells may produce a false positive readout of graft persistence, due to uncoupling of the label from its original host cell[42]. Preclinical genetic labeling of stem cells with fluorescent proteins or bioluminescent enzymes has provided investigators with important information regarding graft behavior in small animal models, offering both fast read-outs of longitudinal cell survival and low costs per imaging study. However, the penetrance of these signals is too low for detection in humans, hence largely limiting their application to small animal models[43]. By contrast, PET and SPECT provide higher sensitivity than do optical techniques, making them better suited for monitoring biological processes in large animal and human studies[17,32,33,44,45], while also sensitively permitting visualization of as low as 1×10^5 engrafted cells[30]. Although spatial resolution remains a limitation with nuclear medicine imaging, it could be overcome by combining with computed tomography[46] (CT) or with MRI. However, the combined PET-CT approach may not be ideal for repetitive assessment of gene or stem cell fate due to the high exposure to ionizing radiation. Hence the combined PET-MRI approach may offer an attractive alternative because of its radiation-free and high spatial resolution qualities[47].

Cell labeling strategies

Labeling cells to enable a combined PET-MRI approach will be of great clinical value for gene and cell therapies. Numerous PET reporter systems have been previously described, including those using dopamine-D2 and somatostatin receptors. However, these systems suffer from low sensitivity, as endogenous receptor expression leads to high background signal[48–50]. The sodium iodide symporter (NIS) has been proposed as an alternative reporter gene due to its wide substrate availability, labeling stability, and well-understood metabolism and substrate clearance[51]. However, the presence of NIS in other tissues such as the thyroid, stomach, and urinary tract reduces its reporter specificity. For PET imaging in gene and stem cell therapy settings, the most widely used label to date has been the *HSV-tk* reporter

gene[17,52–54]. This construct offers several benefits, including quantitative and anatomic evaluation of reporter gene expression[55]. It also has the ability to function as a suicide gene upon administration of exogenous acycloguanosine substrates in pharmacological amounts, making it particularly ideal for ablating unwanted tumorigenic findings[54].

For MRI, the chemical exchange saturation transfer (CEST) as a contrast agent has been used to improve detection sensitivity. It is based on the principle that mobile protons resonate at a frequency distinct from those in bulk fluid. A proton signal specific to a molecule or CEST substrate is selectively saturated with signal. These protons' movement toward, exchange with, and subsequent signal transfer to bulk water results in the exchange transfer of signal loss[56]. While all ^{1}H-based MR reporter genes rely on (super)paramagnetic substances and water relaxation for contrast[57–59], the contrast produced by CEST agents can additionally be switched on and off by frequency selection. Using a lysine-rich protein (LRP) reporter, Gilad et al. demonstrated this principle by creating a contrast material that is detectable in the micromolar range, biodegradable, and capable of distinguishing live from dead cells, thus enabling the constant monitoring of endogenous expression levels in daughter cells[60]. Newer CEST contrast agents such as human protamine-1 address immunogenic concerns regarding the use of animal reporter proteins, and are being investigated for *in vivo* imaging applications[61]. Another advantage is that the separation of signals from different CEST contrast agents enable multiple, simultaneous measurements possible from distinct target populations[62,63]. In contrast to fusion reporter genes, the use of a single reporter gene for multimodal imaging with photoacoustics, MRI, and PET is being explored[64].

Targeted genome editing

Significant advances have been made with respect to genome editing technologies, but these advances have not yet been extensively integrated with bioimaging tools. To date, the majority of genetic engineering to a targeted locus has been accomplished by use of zinc-finger nucleases (ZFNs). More recently, transcription activator-like effector nucleases (TALENs) have offered a similarly promising tool, coupling a generic *Fok*I nuclease domain with a specific DNA-binding domain. For ZFNs and TALENs, DNA-binding modules are engineered to match a target DNA sequence. There, they direct double strand breaks and facilitate potential DNA alterations and repair under non-homologous end joining (NHEJ) or homology-directed repair (HDR). A third genome editing system also offers strong potential for improving targeted gene therapies: the clustered regularly interspaced short palindromic repeats (CRISPR) system uses RNA-guided Cas9 DNAse activity to generate sequence-specific target cleavage[65].

To date, these three genome editing approaches have been used for correction or modeling of α1-antitrypsin disease[66], sickle cell anemia[67], and Parkinson's disease[68]. Recently, Wang et al. demonstrated how ZFN technology can introduce a reporter cassette into the safe-harbor AAVS1 locus of human ESCs and iPSCs with readout capacity by three independent systems: bioluminescence imaging (Fluc), positron emission tomography imaging (HSV-tk), and fluorescence imaging (mRFP)[27]. This work provides a platform for future introduction of a dual reporter gene and corrected cassette under the control of the target site promoter,

providing important insights into temporal and spatial activity of cell fate. Collectively, these three approaches (ZFN, TALEN, and CRISPR) make potent tools for genomic targeting. Investigators now have the ability to (1) guide genomic integration of reporter genes and corrected genes, and (2) to monitor the behavior of edited grafts with bioimaging platforms.

Conclusions

Gene and stem cell therapies, individually or integrated into one therapeutic product, have yet to realize their full potential. Two significant hurdles are (1) a lack of regulatory confidence in the safety and specificity of genomic manipulations in gene correction or in cell differentiation, and (2) a lack of understanding into the long term survival kinetics and behavior of transplanted cells or integrated gene products. We propose here that bioimaging will play a critical role in overcoming these barriers by providing more quantitative and longitudinal readouts of graft and vector behavior, and lead to more informed and comprehensive patient care. The use of bioimaging in an integrated, collaborative approach will offer valuable insight into the delivery, engraftment, survival, and host tissue interactions of vectors and cells, as well as early knowledge of off-target behavior and oncogenic events. By providing powerful tools for guiding clinical practice and scientific development, bioimaging is assured of a bright future as a research field.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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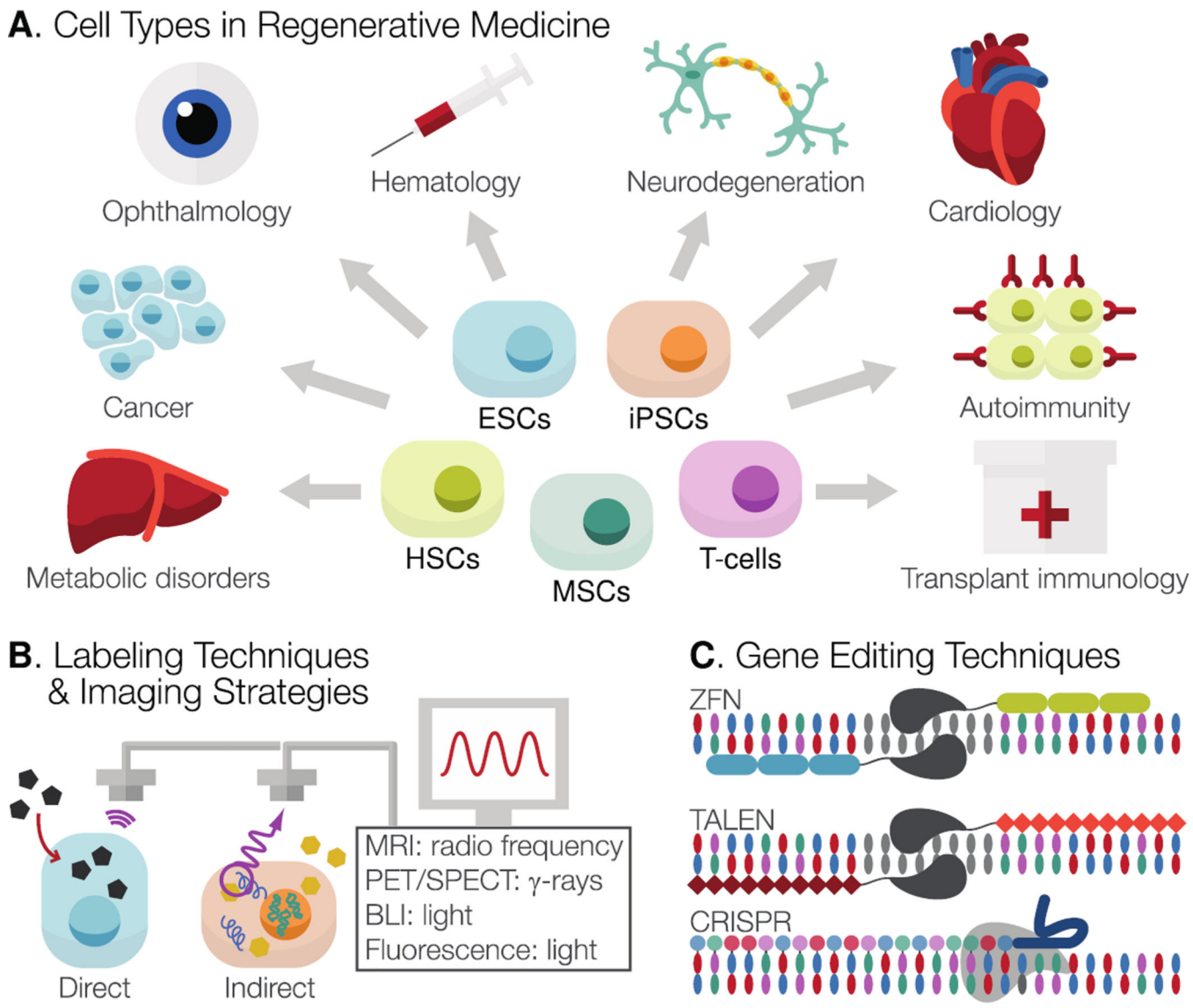

Figure 1. Pathways in gene- and cell-based therapies

(A) A variety of cell types, including somatic cells (HSCs, T-cells) and pluripotent stem cell (ESCs, iPSCs) derivatives, are available to investigators to address a wide range of pathologies across fields. **(B)** To enable *in vivo* monitoring of transplanted cells by protein, enzyme, and receptor-based platforms, cells can be labeled either *"directly"* (with a physical compound such as iron particles or radiotracers) or *"indirectly"* (by genetic integration of reporter gene(s)). **(C)** Targeted genome editing can be achieved by several techniques, including ZFN, TALEN, and CRISPR approaches. Dual editing of cells to integrate corrected gene products with reporter cassettes will facilitate informed assessment of their safety and efficacy by bioimaging. HSCs, hematopoietic stem cells; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; MRI, magnetic resonance imaging; PET/SPECT, positron emission tomography/single photon emission computed tomography; BLI,

bioluminescent imaging; ZFN, zinc finger nuclease; TALEN, transcription activator-like effector nuclease; CRISPR, clustered regularly interspaced short palindromic repeats.

Table 1

Clinical studies using *in vivo* bioimaging for tracking cell fate

Human clinical trials or case reports using “direct” and “indirect” labeling strategies for *in vivo* bioimaging to monitor homing and migration of transplanted cells.

Labeling:	Imaging Modality	Cell type	Treatment	Procedure	Outcome	Source
^{18}F–FHBG	PET/MRI	Cytolytic T-cells (CTL) *(1×10^{9} cells)*	Immunotherapy for glioblastoma multiforme	- Genetically modifying CTLs with *HSV-tk* to target glioblastoma - Administration of CTLs (after tumor resection) with Rickham reservoir	- Tracking of CTLs up to 2 hours after infusion - Possibility to induce programmed cell death of CTLs after ganciclovir administration	17
^{18}F-FDG	PET/CT	Hematopoietic stem cells (HSC) *(4.5×10^{8})*	Cell therapy after myocardial infarction	- HSC isolation via spectra apheresis - 40 min incubation with ^{18}F-FDG - Administration via balloon catheter	- High resolution assessment of retention in myocardium (1.5% of cells) - Short half-life of ^{18}F-FDG limits usefulness after 20 hours	44
SPIO + ^{111}In-oxine	MRI + Scintigraphy	Dendritic cells (DC) *(15×10^{6} cells)*	Immunotherapy for melanoma	- DC isolation from PBMC - 15 min incubation with ^{111}In-oxine - SPIO added to culture media - Injection into draining lymph node of resected area	- Similar sensitivity between scintigraphy and MRI - Scintigraphy allows quantification of cells - MRI superior in assessing location and migration of labeled cells	35
SPIO	MRI	Neural stem cells (NSC)	Cell therapy for brain damage	- NSC isolation from exposed neural tissue - 60 min incubation with SPIO - Stereotactic implantation in region of brain damage	- Non-invasive tracking of engraftment and migration of labeled cells up to 7 weeks	69
^{111}In-oxine	PET	Circulating progenitor cells (CPC) *(10^{6} cells)*	Cell therapy after myocardial infarction	- CPC isolation from PBMC - 60 min incubation with ^{111}In-oxine - Administration via balloon catheter	- Reduced homing of cells in chronic MI (>1 year) versus acute MI (<14 days)	45

Labeling:	Imaging Modality	Cell type	Treatment	Procedure	Outcome	Source
Technetium-99m	SPECT	Bone marrow mononuclear cells (BMMNC) *(~100×10^6)*	Cell therapy for non-ischemic DCM	- BMMNC isolation via apheresis - Labeling of CD34$^+$ cells with Technetium-99m before injection - Intracoronary or transendocardial injection in affected myocardium	- Better homing of cells leads to increase in LVEF at 3 and 12 months after procedure - Superior engraftment with transendocardial injection	[32, 33]

^{18}F-FHBG: Flurorine 18-9-[4-fluoro-3-(hydroxymethyl)butyl]guanine; **^{18}F-FDG:** Fluorine 18-fluorodeoxyglucose; **SPIO**: super paramagnetic iron oxide; **CPC**: circulating progenitor cells; **MI:** myocardial infarction; **^{111}In-oxine**: indium oxine; **Technetium-99m:** ^{99m}Tc-hexamethylpropyleneamine oxime; **DCM**: dilated cardiomyopathy; **HSV-tk**: herpes simplex virus thymidine kinase; **LVEF**: Left ventricular ejection fraction; **PBMC**: peripheral blood mononuclear cells