

# In vivo imaging of embryonic stem cell therapy

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**Abstract** Embryonic stem cells (ESCs) have the most pluripotent potential of any stem cell. These cells, isolated from the inner cell mass of the blastocyst, are “pluripotent,” meaning that they can give rise to all cell types within the developing embryo. As a result, ESCs have been regarded

as a leading candidate source for novel regenerative medicine therapies and have been used to derive diverse cell populations, including myocardial and endothelial cells. However, before they can be safely applied clinically, it is important to understand the in vivo behavior of ESCs and their derivatives. In vivo analysis of ESC-derived cells remains critically important to define how these cells may function in novel regenerative medicine therapies. In this review, we describe several available imaging modalities for assessing cell engraftment and discuss their strengths and limitations. We also analyze the applications of these modalities in assessing the utility of ESCs in regenerative medicine therapies.

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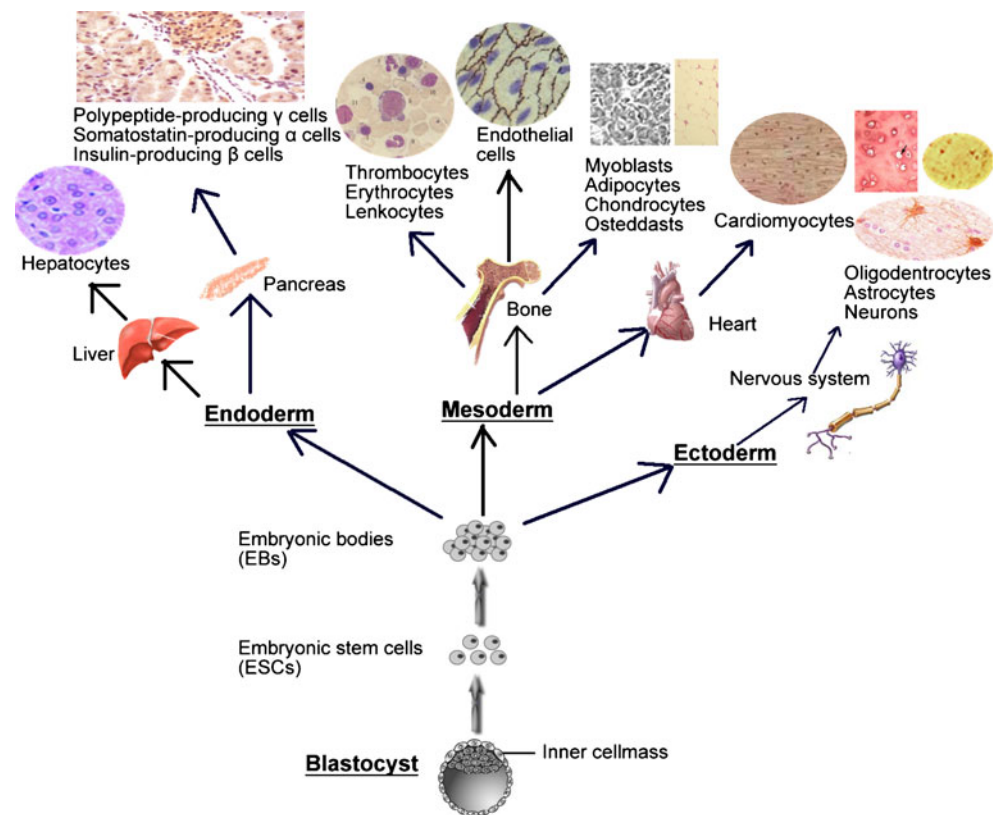
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## Introduction

Embryonic stem cells (ESCs) were isolated from the inner cell mass (ICM) of the mouse preimplantation embryo in 1981 [1, 2]. These cell lines were generated by removing the ICM from preimplantation blastocysts and serially passaging the resulting ESCs on irradiated feeder layers. Two unique properties of ESCs are their unlimited self-renewal capacity and pluripotency; that is, they are able to differentiate into all derivatives of the three primary germ layers: ectoderm, mesoderm, and endoderm (Fig. 1). Additionally, ESCs are capable of propagating themselves indefinitely under defined conditions. This allows them to be used in cell replacement. The first human ESC (hESC) line was established in 1998 from in vitro fertilized embryos [3]. To date, the demonstration of their developmental potential [3, 4] in vitro has evoked widespread

**Fig. 1** The differentiation pathways of embryonic stem cells. Possible differentiation pathways of ESCs are shown. The ESCs can give rise to three germ layers: ectoderm, mesoderm, and endoderm



discussion regarding future applications of hESCs in regenerative medicine.

hESCs have been regarded as a leading candidate source for novel regenerative medicine therapies and have been used to derive various cell populations, such as cardiomyocytes and endotheliocytes. However, before ESCs can be safely applied clinically, their *in vivo* behavior and that of their derivatives must be determined. *In vivo* analysis of ESCs or ESC-derived cells remains critically important to define how these cells function for novel regenerative medicine therapies.

*In vivo* cell tracking offers insight into the underlying biological processes of stem cell-based therapies, with the aim of depicting cell function, migration, homing, and engraftment at organ, tissue, cellular, and molecular levels. Thus, development of novel imaging strategies that can help determine the fate of engrafted cells is expected to have great impact on the understanding of stem cell biology and physiology *in vivo*. The ideal imaging modality should provide integrated information related to the entire process of cell engraftment, survival, and functional outcome, which include the optimal cell type, the number of cells to be delivered, the most suitable route and optimal time point for cell delivery, the biodistribution of the therapeutic cells, and the specific mechanism by which therapeutic cells contribute to functional improvement [5].

Current imaging modalities have been proposed for *in vivo* tracking of the biological fate of stem cells, including

magnetic resonance imaging (MRI), positron emission tomography (PET), bioluminescence imaging (BLI), and fluorescence imaging (FLI) with quantum dots (QDots). In this review, we will discuss each of these *in vivo* imaging techniques with regard to ESCs and their application in regeneration medicine.

## Imaging modalities

### Positron emission tomography (PET)

Two main strategies for *in vivo* stem cell detection using PET have been described: direct imaging and indirect imaging.

Direct imaging—that is, direct cell labeling with a radioactive isotope—has been used for many years to track cells *in vivo*. Cells are incubated with a radiotracer, which allows lipophilic molecules to diffuse and be “trapped” in the cells. After a short incubation period, the cells are washed to remove any unbound activity and then injected into the host. Labeling efficiencies close to 100% are not uncommon and depend on several factors, including cell type, incubation time, environment, and radiotracer characteristics and concentrations [6]. The key drawbacks of direct PET imaging include dilution of signal from cell division and lack of ability to determine cell viability and

function. Furthermore, the imaging technology has too much reliance made on the resolution power of the PET instrument. However, this approach is often used to track stem cells owing to its minimal toxicity and easy accessibility.

Indirect imaging strategies are more complex, and one example of indirect imaging that is now widely used is reporter gene imaging. There are three different types of PET imaging reporter gene strategies (Fig. 2): receptor-based, enzyme-based, and transporter-based reporter gene imaging systems.

### 1. Receptor-based reporter gene imaging system

Radiotracers are ligand molecule probes, which interact with the receptor gene encoded, and then are trapped. Prominent examples are tracers that target the dopamine 2 receptor (hD2R) and somatostatin receptor subtype 2 (hSSTR2).

hD2R is one of the few systems that can be used for cell tracking in the brain because of the ability of the receptor probes, such as  $^{18}\text{F}$ -labeled fluoroethylspiperone (FESP), to cross the intact blood-brain barrier (BBB). Similar to the hD2R system, hSSTR2 is a potential reporter gene for human studies. Octreotide, P829, and P2045 are synthetic somatostatin analogs that bind to somatostatin receptor subtypes 2, 3, and 5 of human, mouse, and rat origin [7]. Therefore, radionuclide-labeled derivatives of somatostatin receptor agonists and antagonists, such as [ $^{64}\text{Cu}$ ]TETA-

octreotide, have been developed and could be used for noninvasive reporter gene imaging [8].

### 2. Enzyme-based reporter gene imaging system

For this approach, an enzyme, the marker gene product, converts a radioactive probe into a metabolite that is selectively trapped in the transfected cell. Thus, the more probes accumulate in the cells, the greater the signal strength detected by PET. A number of reporter genes have been developed, including herpes simplex virus type 1 thymidine kinase (HSV1-tk) and its mutant form HSV1-sr39tk, which are most widely used for PET imaging.

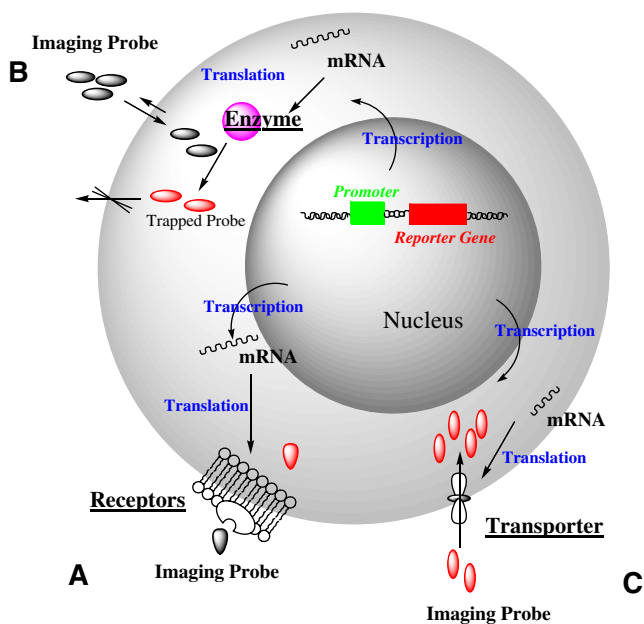
Thymidine kinase phosphorylates include a range of substrates such as thymidine, analogs of pyrimidine, and acycloguanosines. Derivatives of these phosphorylates have been radiolabeled with various isotopes and used for PET, such as  $^{18}\text{F}$ -labeled 9-[3-fluoro-1-hydroxy-2-propoxymethyl]guanine (FHPG) and  $^{124}\text{I}$ -labeled 2'-fluoro-2'-deoxy-5-iodo-1-beta-D-arabinofuranosyluracil (FIAU) [9], among others.

### 3. Transporter-based reporter gene imaging system

Transport proteins can be expressed in cell membranes and have high specificity for certain compounds. For example, the sodium/iodide symporter (NIS) can be visualized with radioactive iodide. NIS has been utilized in combination with  $^{124}\text{I}$  to monitor reporter gene expression using PET. Its long half-life (4.12 days) allows tracking of slow biochemical processes. However, intracellular retention has been found to be low because of the rapid release of radioactive iodide [10].

The above three kinds of reporter probes could be expressed as long as the cells survive and are passed to the daughter cells upon cell division, so reporter genes are useful for detecting the survival of implanted cells. In addition, only viable cells can be detected, because the accumulation of the reporter probes requires expression of the reporter gene products.

The direct and indirect imaging strategies for PET are all utilized widely. Although the value of PET lies in its high-sensitivity tracking of biomarkers in vivo, potential disadvantages of PET include repeated injection of radioactive substances into an organism with the potential for radioactive damage to normal tissues related to radiation accumulation and exposure of surrounding tissues [11]. Additionally, most currently available radiotracers have short half-lives, which limits their use to short-term tracking and has potential adverse effects on stem cell viability and differentiation, and the leakage of the tracer from the labeled cells, which can lead to a false-positive signal [12]. Furthermore, nonspecific uptake of the radiotracers by normal tissue may hamper sensitive detection of stem cells [11, 13].



**Fig. 2** Three types of PET imaging reporter gene strategies. **a** Receptor-based reporter gene imaging system. **b** Enzyme-based reporter gene imaging system. **c** Transporter-based reporter gene imaging system

### Bioluminescence imaging (BLI)

BLI is a technique in which a charge-coupled device camera detects light emitted from a substrate to the enzyme luciferase. It is defined by the *luc* gene and is based on luciferase-mediated oxidation of luciferin (emission spectra: 400–620 nm).

The significant advantage of BLI is its high sensitivity (100–1,000 cells for more superficial anatomical sites). It is safe and permits the repeated tracking of small numbers of labeled cells. Other advantages of BLI include the simplicity of the procedure, the relatively low cost of equipment, the permanent reporter gene labeling (i.e., no dilution of label upon cell division), and the ability to assess whole-body distribution without background signal. However, its very low resolution (in the range of millimeters) is a disadvantage, and another limiting factor is light scattering and absorption by the tissues, which has restricted the application of this technique to small animal models [14]. Furthermore, this technique requires the stable expression of nonhuman genes and the injection of high concentrations of potentially immunogenic nonhuman substrates (e.g., luciferin), which limits its application for human use [6].

### Magnetic resonance imaging (MRI)

MRI is a widely used medical imaging technique in which magnetic fields are used to detect the nuclear spin of molecules. MRI is the most readily accessible method for tracking ESC engraftment. It can provide detailed morphological and functional information and seems ideally suited to integrate efficacy assessments with the capability for cell tracking. MRI has been successfully applied to detect and track stem cell migration, particularly in cardiac [15–18] and brain disease [19–22] models.

Drawbacks of MRI include low sensitivity and difficulty in quantifying a labeled cell population. There is a risk for release of iron oxide after cell death and its accumulation in bystander cells; therefore, the intracellular markers may become diluted with cell division, making it difficult to quantify proliferating cells, and false signals may occur owing to shedding and subsequent sequestration of iron particles [23]. Thus, the imaging signal is not directly linked to cell viability. However, novel direct labeling techniques such as CLIO-Tat peptides [24] or magnetic relaxation switches [25] are under development in preclinical studies and may overcome some of these limitations.

### Fluorescence imaging (FLI) with quantum dots (QDots)

QDots are semiconductor nanocrystals whose size and shape can be precisely controlled by the duration, temper-

ature, and ligand molecules used in their synthesis [26] and have physical properties that enable them to emit fluorescent light from 525 to 800 nm. QDots consist of an inorganic core, a shell of metal, and an outer organic coating layer with a total diameter of 2–10 nm [27]. QDots have a narrow emission and continuous broad absorption spectrum. This reduces autofluorescence and enables single molecule detection, which increases sensitivity and allows multiple QDots (multiplexing) to be visualized with a single light source [28]. Furthermore, reduced photo bleaching and blinking make QDots ideally suited for long-term observations or repeated measurements in vivo [29]. Bioconjugated QDots can be used to track key biomolecules, such as growth factor receptors, integrins, phospholipids, and enzymes, when stem cells are exposed to different environments or soluble factors [30–33].

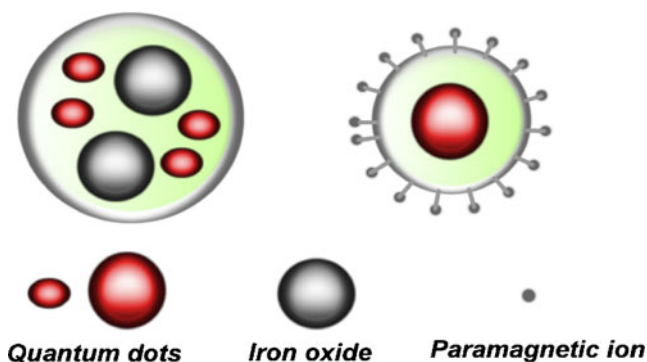
QDots represent a fairly new advance for tracking stem cells. Stem cell survival, differentiation, and function either in vitro or in vivo have been monitored [34–36]. However, cytotoxicity and the potential interference of QDots labeling with cellular processes are primary limitations in any live cell or animal experiment. This method has not yet been used in vivo to track stem cells in animals larger than mice [27].

### Multimodal imaging

Cell tracking demands high sensitivity and spatial resolution from imaging modalities because of the microscopic targets. Multimodal imaging is an important method of cell tracking given the unique strengths that each modality can offer and the potential for corroboration between different imaging modalities as well ex vivo verification [37, 38].

A multimodality reporter system expressing a fusion protein makes the use of a particular imaging technique possible that best suits the application. For example, murine ESCs have been stably transduced with a self-inactivating lentiviral vector containing fluorescence (firefly luciferase; Fluc), BLI (monomeric red fluorescence protein; mRFP), and PET (HSV-tk) reporter genes [39]. Each portion of the multimodality imaging construct offers its unique benefits [12]: mRFP allows isolation of the stably transduced cell population and Fluc enables high throughput in small animal models. In addition, HSV-tk enables PET imaging in small animals [40, 41], large animals [42, 43], and humans [44, 45].

Another type of multimodality imaging is multimodal contrast agents, which are based on highly fluorescent QDots combined with magnetic nanoparticles or ions to form an exciting class of new materials for bioimaging [46] (Fig. 3). Approaches have been developed that integrate fluorescence and magnetic properties in a single nanoparticle. Currently, the combination of QDots with a



**Fig. 3** Magnetic nanoparticles and ions and QDots for stem cells trafficking [46]

radiolabel has shown to be valuable for the *in vivo* detection of the pharmacokinetics and biodistribution of QDots with PET imaging [47]. Although this type or design of particle is the most advantageous or promising for bioapplications, issues remain that must be resolved, for example, the toxicity and the circulation half-time of the particles, and the mechanisms by which they are cleared (kidney, liver, or elsewhere) [46].

Because each imaging modality possesses unique limitations and advantages (Table 1), multimodality imaging constructs have been developed that combine the best features of each technology in an attempt to satisfy all the needs of stem cell imaging. Some examples are discussed below.

### Imaging of regenerative medicine therapies

#### Cardiac diseases

ESC transplantation is being widely investigated as a potential therapy for cell death-related heart diseases. For

example, the death of cardiomyocytes is a key factor in heart failure. Therefore, the formation of new cardiomyocytes could lead to myocardial tissue regeneration and thus represents a putative therapeutic strategy [48]. ESCs could serve as a constant source of cardiac cells. Several groups have shown that murine ESCs can improve cardiac function in mice and rats following an ischemic event [49–52]. However, those studies relied on invasive technologies, such as green and cyan fluorescence, which required the animals to be killed. Molecular imaging would allow *in vivo* tracking of cells and can provide a better understanding in the evaluation of the functional effects of ESC-based therapies. Ebert et al. [16] used MRI to detect and follow transplanted mouse embryonic stem cell (mESC)-derived cardiomyocytes over a period of 28 days in mice using an established myocardial infarction (MI) model. The opportunity to simultaneously acquire data about cardiac structure and function, as well as the cell-tracking capabilities, afforded by MRI is the major advantage of this approach. The use of delayed enhancement MRI with gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) on day 1 post-MI showed that infarct size was similar between the treated and untreated groups. The transplanted mESCs loaded with superparamagnetic microspheres were detected along the borders of the infarct zone and remained in and around the infarcted region throughout the 28-day evaluation period. Long-term follow-up studies using noninvasive assessment are needed to better define whether a cell and its progeny can contribute to cardiac engraftment and repair.

Cao et al. [53] used BLI to track human embryonic stem cell (hESC)-derived cardiomyocyte implantation in the post-MI heart for 8 weeks. They found loss of more than 90% of the donor population within the first 3 weeks of delivery after the administration of  $1 \times 10^6$  hESC-derived cardiomyocytes and that stable grafts continued to form for

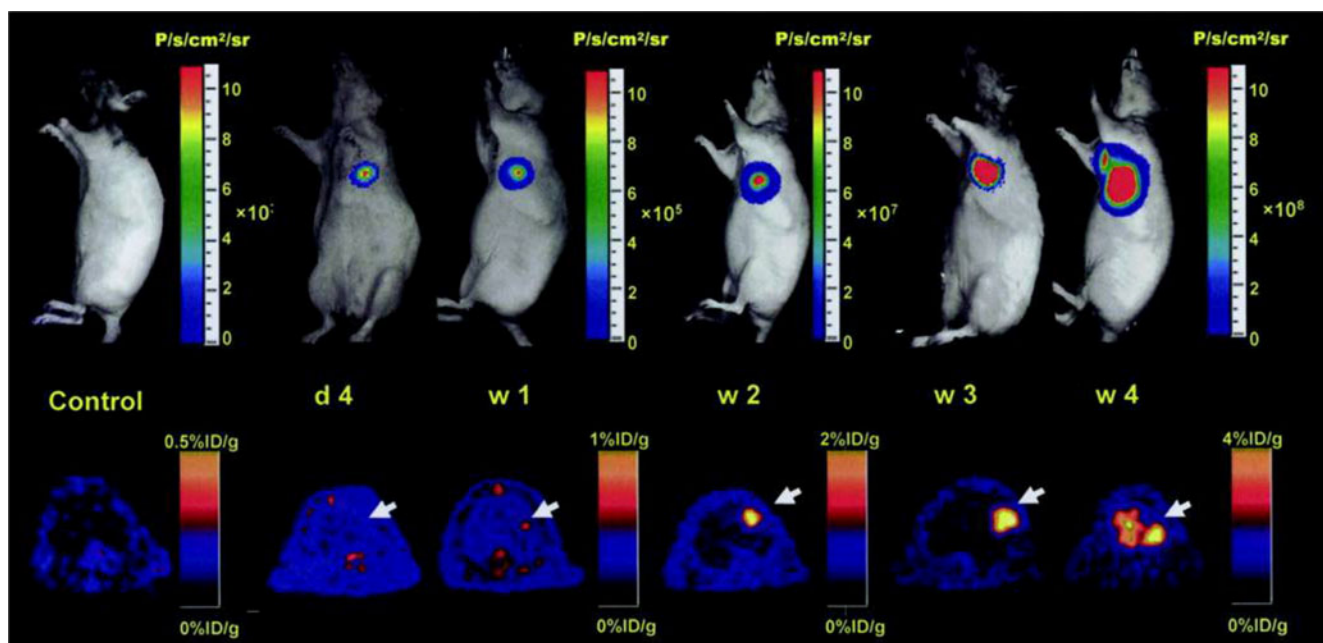
**Table 1** Characteristics of imaging modalities

	BLI	MRI	PET	FLI
Resolution	3–5 mm	25–100 $\mu$ m	1–2 mm	2–3 mm
Sensitivity	$10^{-15}$ – $10^{-17}$ mol/l	$10^{-3}$ – $10^{-5}$ mol/l	$10^{-11}$ – $10^{-12}$ mol/l	$10^{-9}$ – $10^{-12}$ mol/l
Visualization	2D	3D	3D	2D
Penetration depth	1–2 cm	No limit	No limit	1–2 cm
Cell tracking	Small animal	Any model	Any model	Small animal
Stability	Weeks	Weeks	Minutes–days	Weeks
Radiation	No	No	Yes	No
Quantifiable	Yes	No	Yes	No
Advantages	Highest sensitivity; quick, easy, relatively low cost of equipment	Highest spatial resolution; morphological and functional imaging	High sensitivity; quantitative; signal directly related to cell viability	Broad excitation profiles; narrow emission spectra; multiple wavelengths/colors
Disadvantages	Low spatial resolution; only for small animals	Low sensitivity; not directly reflecting viable cells	Lack of anatomical information; radiation to subject; short-life of radioisotopes	Potential toxicity; induce cell death; limited to small animals

more than 6 months. Thus, the acute donor cell death that occurred in the initial weeks after engraftment suggests that hESC-derived cardiomyocytes contributes only temporarily to improved systolic function in cell recipients [54]. Similar results showing drastic reductions of signal intensity within the first 1–4 days after transplantation were noted by Wu et al., among others, using BLI and PET imaging, possibly because of the acute donor cell death from inflammation, ischemia, and apoptosis as shown on histology [41, 55, 56]. To better understand the fate of ESCs in vivo, multiple reporter genes can also be introduced into the same cell for multimodality imaging.

Hung et al. [17] transplanted dual-labeled mESCs with superparamagnetic iron oxide (SPIO) and luciferase into myocardial infarction mice. They directly injected labeled mESCs into three different zones: intra-infarction, peri-infarction, and normal (remote) after left anterior descending artery ligation. Their results as assessed by molecular reporter gene imaging technique demonstrated that BLI activity significantly increased over time and that mESCs transplanted into different zones of MI significantly altered cell survival. This observation suggested that the transplanted mESCs proliferated. Similarly, Qiao et al. [18] used MRI and PET dual detection of cardiac grafted ESCs to assess survival and proliferation of ESCs in normal and infarcted myocardium. In that study, murine ESCs were stably transfected with mutant *HSV1-sr39tk* and also were labeled with SPIO particles. ESC-treated rats have improved

global and preserved regional left ventricular (LV) function because of favorable ventricular remodeling after MI. Additionally, they found that SPIO labeling permitted visualization of detailed cell distribution by using MRI at day 1, a few days before the 9-(4-fluoro-3-hydroxymethylbutyl) guanine (FHBG) signals became detectable. More specifically, Cao et al. [40] reported characterizing ESCs that stably express fluorescence, BLI, and PET reporter genes and monitoring the kinetics of ESC survival, proliferation, and migration. Murine ESCs were stably transduced with a lentiviral vector carrying a novel triple-fusion (TF) reporter gene consisting of firefly luciferase, monomeric red fluorescence protein, and truncated thymidine kinase (fluc-mrffp-ttk). Results of that study suggested that BLI and PET signals increased progressively during the initial 4 weeks (Fig. 4), which indicated ESC survival and proliferation in the host. The fluc and ttk reporter genes that are linked and expressed as fusion proteins may allow a robust correlation between BLI and PET results via quantification of cell signal activity. Furthermore, the results demonstrated that TF reporter gene did not affect ESC differentiation. Despite the conceptual promise of this method, the use of multiple reporter gene imaging to monitor ESC engraftment remains limited so far to animal studies. However, even given the limitations, using these imaging tools to determine the transplantation and assessment of ESC survival and proliferation in vivo in a myocardial ischemia model can indicate the effect of ESCs on the reparation of cardiac function.



**Fig. 4** BLI and PET imaging of the transplanted ESCs survival. A representative study mouse injected with murine ESCs that stably transduced with a lentiviral vector carrying a novel triple-fusion (TF) reporter gene consisting of firefly luciferase, monomeric red fluores-

cence protein, and truncated thymidine kinase (fluc-mrffp-ttk) showed significant BLI (*top*) and PET (*bottom*) signals at day 4, week 1, week 2, week 3, and week 4 when longitudinal cell survival was assessed. By contrast, control animals had only background activities [40]

## CNS disorders and diseases

Stem cell therapy is particularly promising for restoring impaired functions in numerous damaged brain areas after ischemia, seizures, and traumas or in diverse degenerative disorders [48, 57]. Long-term graft survival, maintenance of cellular phenotype, and the capacity of the graft to functionally interact with the host tissue are essential parameters for *in vivo* success. For example, Mimeault and Batra [48] suggested that optical BLI can serve as an essential tool for studying the development of hESC-based grafting strategies in the CNS.

### Ischemic diseases

A number of factors that contribute to the loss of neurons after ischemia have been described. Under certain conditions, short periods of brain ischemia cause delayed cell death, and neurons, stressed by raised intracellular calcium and the other molecular events that occur in ischemic stroke, are initiated to die. Stem cell transplantation may not only prevent ischemic damage but also actually repair the injured brain [58]. A variety of human cell types have been tested in experimental stroke [59], including neural stem/progenitor cells (NPCs), hematopoietic/endothelial progenitors, and stromal cells isolated from bone marrow. hESCs can be differentiated into NPCs by various methods [60–62]. Several groups have reported that enhanced recovery resulted from engraftment into the stroke brain using different preparations of hESC-derived NPCs [60, 63, 64]. Evidence to date suggests that continued development of ESC therapies may ultimately lead to viable treatment options for ischemic brain injury. However, for monitoring the transplanted cells, *in vivo* imaging will also be necessary to determine the response of the brain to cell therapy. Functional imaging studies, including PET (to monitor metabolic activity), functional MRI (to monitor cerebral plasticity), and MRI tracking of macrophages that have phagocytosed ultrasmall SPIO particles (to monitor brain inflammation), will be used as clinical indicators of graft efficacy [58].

It has been reported that transplantation of undifferentiated ESCs into an intact animal can cause teratomas or even highly malignant teratocarcinomas [3, 65] but that when cells are predifferentiated *in vitro* before implantation, the tumorigenic potential of ESCs seems to be greatly reduced. Using MRI detection on ESC migration in an experimental stroke model of rat, Hoehn et al. [21] made the surprising observation that transplantation of murine ESCs did not produce tumors even in the absence of any predifferentiation. Instead, ESCs migrated across the midline toward the surrounding peri-infarct and began to differentiate into neural precursor cells on the way to the lesion [66]. More

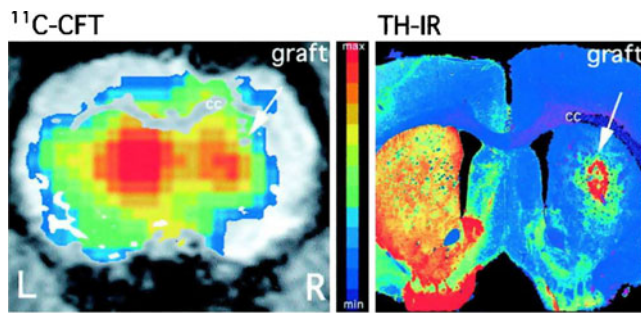
specifically, real-time imaging of transplanted ESCs is essential for understanding their interactions *in vivo* with host environment. Daadi et al. [22] used BLI and MRI to visualize the fate of grafted hESC-derived human neural stem cells (hNSCs) in stroke-damaged rat brain. The hNSCs stably expressed enhanced green fluorescence protein and firefly luciferase reporter genes. Cell survival was tracked noninvasively by MRI and BLI for 2 months after transplantation and confirmed histologically. It was shown that grafted hNSCs differentiated into neurons, into oligodendrocytes in stroke regions undergoing remyelination, and into astrocytes, extending processes toward stroke-damaged vasculatures. Furthermore, their results suggested that the combination of BLI and MRI modalities provides reliable, real-time monitoring of cell fate.

### Parkinson's and Alzheimer's diseases

The progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta, a region of brain that controls muscle movement, could result in motor dysfunctions that are associated with Parkinson's disease (PD). Therefore, stem cell-based therapy delivering new DA neurons has the potential to be a primary strategy for the treatment of this neurodegenerative disease. Alzheimer's disease, which is characterized by regional neuronal degeneration and synaptic loss and is associated with the presence of senile plaques, is another degenerative disorder whose patients could benefit from neural cell delivery in the brain [48, 67, 68]. The best-characterized stem cell population so far is ESCs, including those from humans, which can be differentiated into DA neurons in animal models of PD.

Early reports by Bjorklund et al. [69] showed that transplantation of low doses of undifferentiated mouse ESCs into the rat striatum resulted in a proliferation of ESCs into fully differentiated DA neurons, which caused gradual and sustained behavioral restoration of DA-mediated motor asymmetry. They used PET and  $^{11}\text{C}$ -labeled 2 $\beta$ -carbomethoxy-3 $\beta$ -(4-fluorophenyl)tropane ( $^{11}\text{C}$ ]CFT) to obtain parallel evidence of DA cell differentiation *in vivo* (Fig. 5). Behavioral recovery of rotational asymmetry was determined at 9 weeks after implantation of ESCs in animal models. Furthermore,  $^{11}\text{C}$ ]CFT binding in the grafted striatum increased by 75–90% of the intact side. These findings of efficient ESC transplantation, expansion, and differentiation into functional DA neurons in the brain implicate that ESCs could become a donor source for cell therapy in PD.

Recently, in another study [70] using BLI to monitor the long-term viability and proliferation of hESC-derived neural precursor grafts in the brains of immunodeficient and immunocompetent mice, it was observed that initial



**Fig. 5** The specific dopamine transporter (DAT) ligand [ $^{11}\text{C}$ ]CFT binding in the right grafted striatum was detected using PET, as shown in this brain slice (*left*). Increased [ $^{11}\text{C}$ ]CFT binding was found in the right striatum, which may correlate with the postmortem presence of TH-immunoreactive (IR) neurons in the graft (*right*). Color-coded (activity) PET images were overlaid with MRI images for anatomical localization [69]

signal increased in the immunocompetent mice given grafts of hESC-derived neural precursors, followed by dramatic signal losses between days 10 and 18. The signal attenuation was interpreted as compatible with early graft rejection. These results also demonstrated that there was no significant alteration in the viability of transduced hESC-derived neural precursors in immunodeficient models over a 2-month period, whereas variations in proliferative activity were observed among grafted animals.

Suitable noninvasive imaging modalities may serve as tools for probing neural graft behavior in the living brain, complementing traditional histology-based methods. In particular, using serial BLI and reporter gene technologies can monitor ESCs graft viability and proliferation in the brain during long-term study.

## Diabetes

Type 1 diabetes is caused by the autoimmune destruction of insulin-producing  $\beta$  cells within the pancreatic islets. Islet transplantation is now well established and has allowed some patients to wean off of insulin treatment. However, at present the chronic shortage of organ donors—and thus of available islet cells—is the major limiting factor. One approach to overcome the problem of insufficient supply is to generate islets from proliferative stem cell populations. Currently, in order to realize this goal, the only stem cell population with sufficient proliferative capacity is hESCs, which proliferate in culture at a rate of >250 population doublings per year [71–73]. The pluripotency of hESCs has provided definitive endoderm cells [74], foregut, pancreatic, and endocrine precursor cells [75], and ultimately insulin-secreting cells [75].

One of the most promising approaches is based upon the generation of insulin-producing cells (IPCs) from ESCs. A

number of recent reports have claimed the successful differentiation of ESCs into IPCs [76–78]. Yet a potential drawback of these studies has been the lack of real-time noninvasive monitoring of the transplanted IPCs in vivo. It is extremely important to be able to monitor the fate of transplanted ESC-derived IPCs in vivo noninvasively. Chan et al. [79] developed one such real-time noninvasive BLI modality in recipient mice. Using pancreatic  $\beta$  cell-specific promoters, this approach is based upon the detection of rat insulin promoter (RIP) driven luciferase expression and permits the detection of early events underlying graft survival and function in vivo. Their data indicate that BLI has the potential for the real-time noninvasive monitoring of the fate of the transplanted IPCs, graft survival, and functioning in vivo. However, advances in understanding the mechanism underlying pancreas development after ESCs or ESCs-derived IPCs, tracked by noninvasive imaging, are necessary to achieve further clinical benefit.

## Future perspective

Human ESCs can be induced to differentiate into a variety of cell populations and used for therapeutic applications in regenerative medicine. Recently, the US Food and Drug Administration (FDA) has approved the first phase 1 clinical trial using hESCs for neural regeneration in spinal cord injury patients [80]. However, a major drawback for the generation of hESCs is obtaining a sufficient number of donated oocytes to produce an hESC bank, which obviously raises ethical concerns [81]. In addition, tissue rejection following transplantation in patients will be a difficult hurdle to be overcome. These problems have prompted efforts to reprogram somatic cells back to a pluripotent state, which resulted in the generation of induced pluripotent stem (iPS) cells that are functionally similar to ESCs.

It is widely accepted that mouse and human iPS cells possess morphological, molecular features closely similar to blastocyst-derived ESCs [82].

Because of their pluripotency and therapeutic potential in cell replacement therapy, iPS cells hold a great deal of promise in regenerative medicine. Some groups have already demonstrated the therapeutic potential of iPS cells in neural diseases [83] and cardiovascular diseases [84, 85]. iPS cells are valuable not only for cell replacement therapy but also for disease modeling in vitro, which facilitates studies of mechanisms underlying disease development, drug screening, and development of new therapeutic strategies [86].

To realize the new therapeutic strategies from basic research into clinical application, it is necessary to improve molecular imaging techniques. For example, new reporter



gene systems are under development for PET imaging. It was reported that the estrogen receptor ligand binding domain (ERL)/16 $\alpha$ -[<sup>18</sup>F]fluoro-17 $\beta$ -estradiol (<sup>18</sup>F-FES) is a new in vivo reporter gene imaging system to monitor transduced ESCs in mice [87, 88].

Furthermore, with the combination of PET and CT for small animal research, PET/MRI might soon be available, which may provide new opportunities to study the physiology and anatomy of diseases [89]. “Opti-PET” instrumentation that will combine advantages of optical imaging with high resolution and quantitation ability of PET are also under development [6, 90].

## Conclusion

ESCs are capable of differentiation into all cell types and have regenerative potential in a variety of diseases. Noninvasive imaging techniques have proved to be of tremendous value in preclinical studies for in vivo tracking of transplanted ESCs and measurement of effect of therapy, and such imaging allows for longitudinal, repetitive, and quantitative assessment of transplanted cell survival, proliferation, and migration in vivo. These techniques will also boost the clinical application of ESCs therapy. Although none of these imaging modalities fulfill all of the requirements needed for stem cell therapy research at this time, their use will continue to enable significant advances in understanding stem therapy biology and its mechanisms.

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**Conflicts of interest** None.

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