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Noninvasive *in vivo* cell tracking using molecular imaging: A useful tool for developing mesenchymal stem cell-based cancer treatment

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Abstract

Mounting evidence has emphasized the potential of cell therapies in treating various diseases by restoring damaged tissues or replacing defective cells in the body. Cell therapies have become a strong therapeutic modality by applying noninvasive *in vivo* molecular imaging for examining complex cellular processes, understanding pathophysiological mechanisms of diseases, and evaluating the kinetics/dynamics of cell therapies. In particular, mesenchymal stem cells (MSCs) have shown promise in recent years as drug carriers for cancer treatment. They can also be labeled with different probes and tracked *in vivo* to assess the *in vivo* effect of administered cells, and to optimize therapy. The exact role of MSCs in oncologic diseases is not clear as MSCs have been shown to be involved in tumor progression and inhibition, and the exact interactions between MSCs and specific cancer microenvironments are not clear. In this review, a multitude of labeling approaches, imaging modalities, and the merits/demerits of each strategy are outlined. In addition, specific examples of the use of MSCs and *in vivo* imaging in cancer therapy are provided. Finally, present limitations and future outlooks in terms of the translation of different imaging approaches in clinics are discussed.

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Core Tip: There is substantial evidence of the potential of cell therapies in treating various diseases including cancers. Molecular imaging has been actively used for decades to assess cellular processes, evaluate the properties of certain drugs, screen compound libraries, and visualize the fate of cells. This review aimed to confirm whether noninvasive *in vivo* cell tracking in combination with molecular imaging could be used as a tool for the development of mesenchymal stem cell-based cancer treatment. To that end, the following aspects are outlined in the text: labeling approaches, imaging modalities, advantages and disadvantages of each strategy, and scope and limitations of the various imaging approaches. In conclusion, together with long-term monitoring, a lot can be learned with regard to the hidden potential of MSCs as well as their variable fate in humans.

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INTRODUCTION

Cell-based therapy and *in vivo* imaging

Cell therapies are becoming increasingly popular because of their ability to restore or replace damaged tissues, thereby directly impacting disease progression. Cell-based therapies can be developed with the use of any cell type including primary, stem, immune, or progenitor cells. Various cell-based therapies are presently being tested at the preclinical level. Some of them have even reached clinics (*e.g.*, hematopoietic stem cell transplantation for blood disorders^[1]) and are awaiting approvals^[2]. The translation of cell therapies holds great promise for promotion in the field of regenerative medicine.

Mesenchymal stem cell (MSC) therapy has been particularly successful in preclinical models. The timing of MSC administration has been found to be among the key factors in the determination of the efficacy of cell-based therapies^[3]. Evidence proposes that even though MSCs are retained in certain sites, such as the lungs, intestine, and lymph nodes^[4], they have a role in macrophage recruitment to the sites of injury and enhancing wound healing through paracrine effects^[5]. As with numerous other diseases, MSC therapy has the potential to treat cancerous diseases through migration to the tumor microenvironment. While several studies have exhibited an inhibitory effect of MSCs on full-grown tumors^[6,7], MSCs are also involved in the progression of some cancers^[8,9]. The exact function of MSCs in cancerous diseases is controversial and warrants further investigation.

A combination of cell-based therapies and imaging has revolutionized the field of medicine over the last two decades. Better efficacy of cell-based therapies can be achieved through noninvasive monitoring of transplanted cells with the help of molecular imaging tools. In particular, *in vivo* imaging has become an essential tool for monitoring disease status in longitudinal studies^[10]. It is exhaustively utilized to study cancerous diseases, autoimmune disorders, neurological diseases, and cardiovascular diseases. With the use of *in vivo* three-dimensional (3D) imaging, different biological processes including gene expression, protein trafficking, and cell migration/homing/tumor infiltration can be visualized using high resolution^[11]. For studies involving animal models, readouts can be acquired with the use of the same animal over time, thereby reducing the sample size and discrepancies in measurements^[12]. Implementing *in vivo* imaging at the preclinical level can save a lot of time, money, and resources, which can then be invested in clinical studies. Also, based on preclinical outcomes, scientists and physicians can make informed decisions with regard to the

rapid translation of these approaches to the clinical level^[13,14].

For a particular study, it is essential to consider whether *in vivo* imaging would be superior to other approaches including conventional histopathology, which might be the gold standard for animal studies. Nonetheless, histopathology is a time-consuming and labor-intensive process. Errors can occur during the sampling of tissue and subsequent processing, thereby presenting a bias in studies^[15]. Small animals utilized for *in vivo* preclinical imaging can also pose some challenges in terms of handling, maintaining their physiological balance, administering an appropriate dose of anesthetic, and protecting them from radiation^[15]. Overall, *in vivo* imaging is much faster and is better equipped to capture dynamic interactions between administered cells and its targets without having to sacrifice the animal. On the other hand, histopathological analyses can uncover unique information that imaging platforms may overlook. In most cases, a combination of these two approaches is utilized to confirm the findings and overcome the flaws correlated with each modality.

It is crucial to choose the optimal strategy for *in vivo* imaging, depending upon the research question you are trying to answer. Some imaging modalities offer high resolution and others provide high sensitivity. The cost should also be taken into account because imaging platforms can be very expensive to set up. The strategy should be selected carefully to avoid possible interference with the animal's physiology to acquire accurate as well as reproducible results. Some frequently utilized approaches including nuclear imaging, optical imaging, and magnetic resonance imaging are discussed later in this review along with their advantages and disadvantages. Preferably, an imaging tool should be highly specific and sensitive in tracking cell viability, cause minimal or no toxicity to cells, and allow long-term monitoring, the characteristics that are impossible to be achieved through the use of a single approach^[16]. Thus, multimodal imaging approaches, where two or more imaging modalities are combined to achieve the best results, are widely utilized^[17].

LABELING OF MSCS WITH IMAGING REPORTERS

MSCs are inhomogenous adult stem cells and have the ability to differentiate into any cell type with a mesodermal origin^[18,19]. MSCs account for 0.01% of the total mononuclear cells found in the bone marrow^[20]. Major sources of MSCs are bone marrow, fat, cord blood, and dental pulp^[21]. Cells are first isolated from these sources and then expanded *in vitro* prior to administration to a patient. MSCs are also often manipulated with the use of *ex vivo* viral-vector-mediated gene modifications^[22]. The advantage of working with MSCs is their low immunogenicity^[23], thereby suppressing rejection by the host immune system. The efficacy of MSC therapy can be improved by reprogramming or manipulating the cells to overexpress the gene of interest and utilize them for the targeted delivery of therapeutic proteins to the desired area^[24].

Labeling MSCs and tracking them *in vivo* with the use of noninvasive imaging tools have proven to be a powerful strategy in the last few decades. This approach can yield a lot of useful information relating to the overall stem cell behaviors such as migration pattern, retention capacity, and immune clearance of cells^[16]. MSCs can be labeled either directly through their conjugation with probes or indirectly through the introduction of exogenous reporter genes. Labeled cells can then be visualized with the use of imaging modalities including optically charged coupled devices, positron-emission tomography (PET), magnetic resonance imaging (MRI), and single-photon emission computed tomography (SPECT).

Direct labeling

In the direct labeling approach, different labeling agents including quantum dots (QDs)^[25], fluorophores^[26], radionuclides^[27], and superparamagnetic iron oxide particles (SPIO)^[28] can be utilized to tag MSCs, which can then be imaged after their transplantation into living organisms. In this strategy, labeling agents serve as surrogate markers for the number of stem cells (Figures 1, 2A and 3).

Fluorophores, especially QDs, are widely utilized for *in vivo* tracking of MSCs in longitudinal studies. QDs emit fluorescence upon excitation, which can be achieved with a range of different wavelengths. Some of the merits of labeling cells with QDs include high sensitivity and excellent resolution^[29]. QDs have been utilized to label and monitor MSCs^[30,31] either through passive incubation or targeting peptide-mediated delivery, such as the one used with the Qtracker labeling kit^[25,32]. QDs have been found to be biocompatible in studies performed with the use of *in vitro* models, and QD-labeled MSCs were integrated successfully in co-culture systems^[32]. Besides QDs,

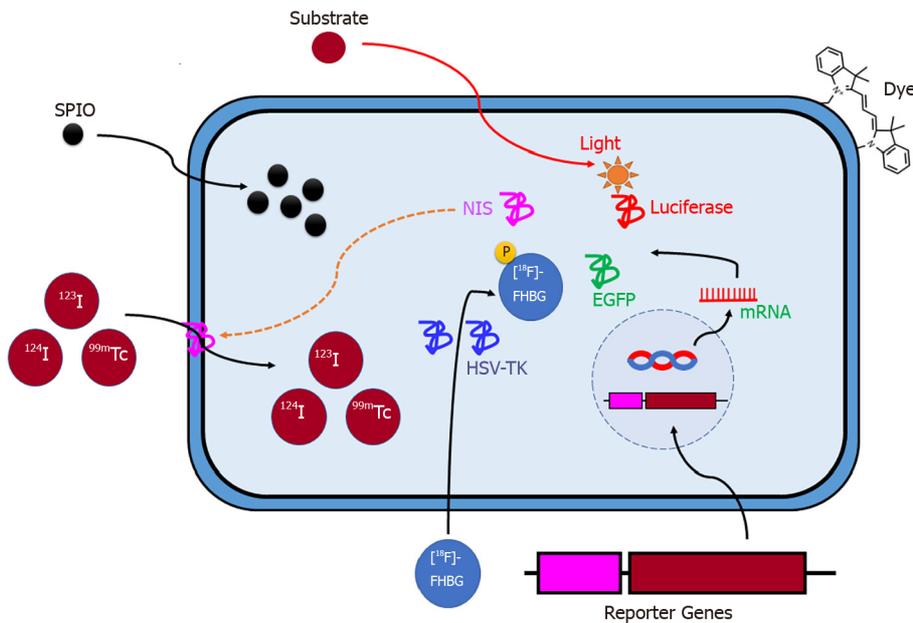


Figure 1 Schematic illustration of labeling mesenchymal stem cells for *in vivo* non-invasive imaging. SPIO: Small superparamagnetic iron oxide; NIS: Sodium iodide symporter; HSV-TK: Herpes simplex virus-thymidine kinase; EGFP: Enhanced green fluorescent protein; [^{18}F]FHBG: 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl) guanine.

lipophilic fluorescent tracer dye such as DiD is another molecule that causes low cytotoxicity to MSCs and can give better contrast^[33]. In recent years, near-infrared (NIR; 700-1000 nm wavelength) fluorophores have been utilized in the clinical setting due to their ability to penetrate deeply located tissues (penetration depth: approximately 4-10 cm), although cardiac imaging is still a challenge. In a previous study, the authors labeled MSCs with the NIR fluorophore IR-786 and delivered them to the coronary artery in a swine model of myocardial infarction. MSC behavior was then successfully monitored 90 min post-injection^[34].

Radionuclides can also be utilized to label cells prior to transplanting them into living subjects. Some examples of radioisotopes are Technetium-99m ($^{99\text{m}}\text{Tc}$; half-life 6 h), Indium-111 (^{111}In ; half-life 2.8 d), Fluorine-18 (^{18}F ; half-life 109 min), and Copper-64 (^{64}Cu ; half-life 12 h). Depending on their physical half-lives, cells labeled with these isotopes can be tracked using imaging tools over a timeframe, ranging from hours to days. Zirconium-89 (^{89}Zr ; half-life 3.3 d) is becoming popular because of its long half-life, thereby allowing long-term monitoring of MSCs with PET imaging^[35]. The biological half-life of radioisotopes should also be taken into account while choosing a suitable radiotracer for the study^[36]. Several studies have reported labeling of MSCs with isotopes including ^{111}In , ^{18}F -fluoro-deoxyglucose (^{18}F -FDG), 2'- ^{18}F -fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil (^{18}F -FEAU), ^{131}I -2'-fluoro-2'-deoxy-1-beta-D-arabinofuranosyl-5-iodouracil (^{131}I -FIAU) and $^{99\text{m}}\text{Tc}$ -D,L-hexamethylene-propyleneamine oxime ($^{99\text{m}}\text{Tc}$ -HMPAO), and subsequent monitoring of cells using imaging strategies including SPECT or PET^[37-40]. Interestingly, previous reports have shown long-term monitoring of MSCs *in vivo* up to 10-14 d when the cells were labeled with ^{111}In ^[41,42].

Labeling of MSCs with SPIO enables *in vivo* tracking of cells by modifying T2 relaxivity^[43,44]. Approaches including electroporation or liposome-mediated delivery can be utilized to introduce SPIOs into cells, which can then be transplanted into the living organism and imaged using high-resolution MRI^[45]. Earlier studies showed the usefulness of this approach in the *in vivo* visualization of MSCs in real time and functional studies involving internal organs^[45,46]. Increased cellular uptake of SPIOs and marginal cytotoxicity was noted with the incorporation of a transfection agent at an optimal concentration^[47]. Alternatively, magneto-electroporation, a new technique that stimulates SPIO endocytosis, can be utilized to quickly label MSCs without transfection agents. This technique has previously been utilized to track MSCs in a peripheral arterial disease rabbit model^[48]. Another study imaged SPIO-labeled MSCs in rats and indicated that these cells may promote the healing of the tendon-to-bone tunnel 4-8 wk post-surgery^[49]. In a brain injury rat model, it was shown that MSCs remain at the lesion site for more than 30 d, as visualized by SPIO labeling and MRI,

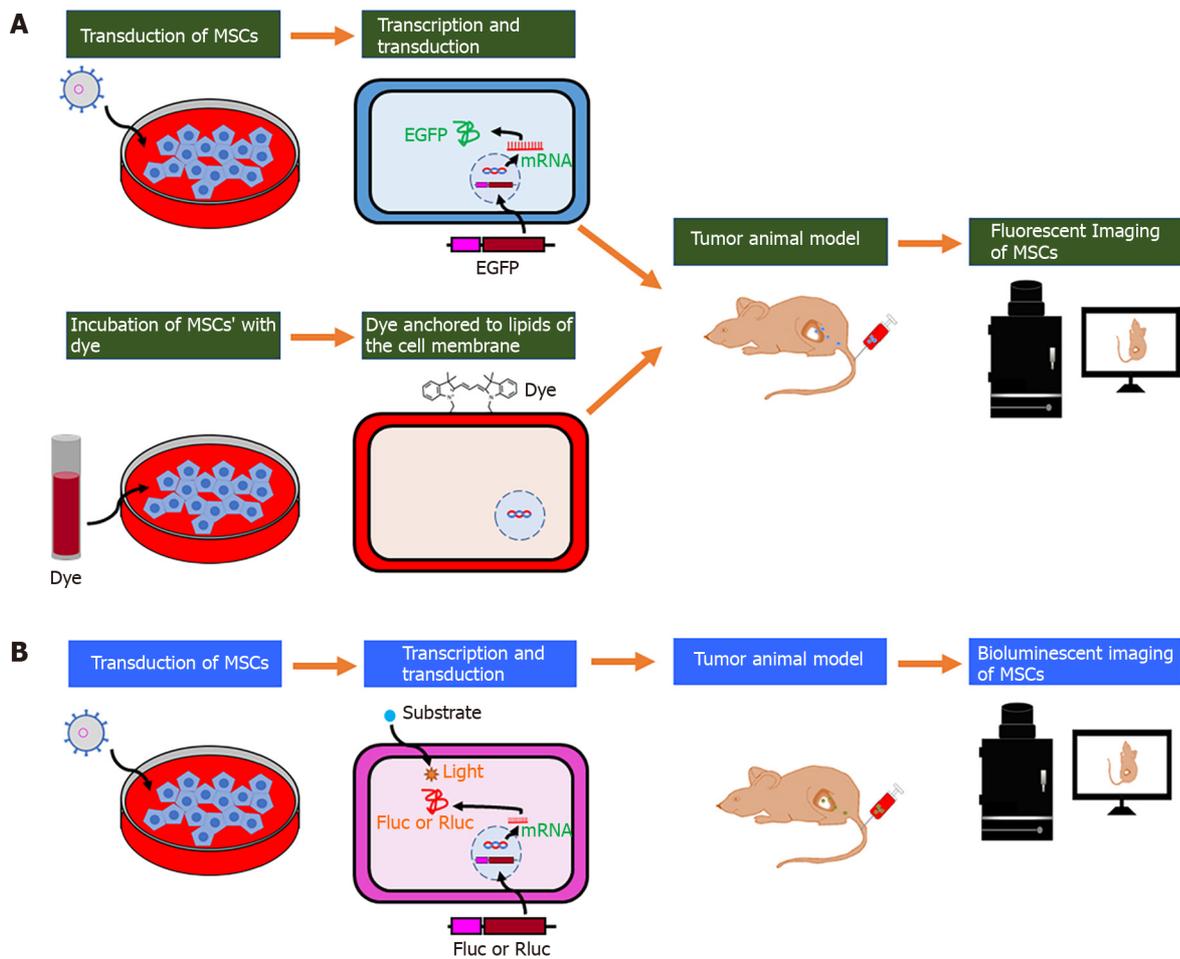


Figure 2 Schematic illustration of the labeling strategy for *in vivo* tracking of mesenchymal stem cells by optical imaging. A: After fluorescent protein (enhanced green fluorescent protein) transduction into mesenchymal stem cells (MSCs) or binding of lipophilic labeling agents (e.g., fluorescent nanoparticles and VivoTrack 680) to the membrane of MSCs, cells are injected into the tumor-bearing mice, and their migration is visualized with the use of *in vivo* fluorescent imaging; B: After the bioluminescent protein (Firefly or Renilla luciferase) transduction into MSCs, cells are injected into the tumor-bearing mice. The light emitted due to the interaction between luciferase and its substrates (D-luciferin or coelenterazine) is captured by *in vivo* bioluminescent imaging. MSCs: Mesenchymal stem cells; EGFP: Enhanced green fluorescent protein; Fluc: Firefly luciferase; Rluc: Renilla luciferase.

and improve survival^[50]. A previous report indicated the use of ferumoxytol, an ultra-small SPIO nanoparticle (USPION), to monitor MSCs by MRI^[51]. USPIONs are a class of coated nanoparticles with various applications. Ferumoxytol, a Food and Drug Administration (FDA)-approved molecule, has shown promising results for imaging MSCs in mouse models, showing its potential use in future clinical trials.

The direct labeling approach can also be utilized with multimodal imaging. A previous study utilized mesoporous silica nanoparticles to label MSCs, which were delivered to an orthotopic U87MG glioblastoma model, and tracked the labeled MSCs with the use of a multimodal approach through the combinatorial imaging of MRI, fluorescence, and PET^[52]. USPION labeling did not modify the differentiation potential or protein content of MSCs^[53]. Imaging indicated that particles loaded into MSCs were quickly taken up by tumor cells and had long retention time in comparison with those used alone^[52].

Indirect labeling

Cells can be labeled indirectly through the stable or transient expression of exogenous reporter genes. This approach improves our understanding of stem cell biology and associated mechanisms by allowing us to study the stem cell behavior over time following transplantation in living subjects (animal models of different diseases or humans)^[54,55]. Incorporated reporter genes are transcribed and translated in viable cells, resulting in the expression of proteins, which interact with suitable substrates to generate a signal that can then be captured using different imaging modalities (Figure 2B and 3A).

Reporter genes including Firefly luciferase (Fluc) and Renilla luciferase (Rluc) in

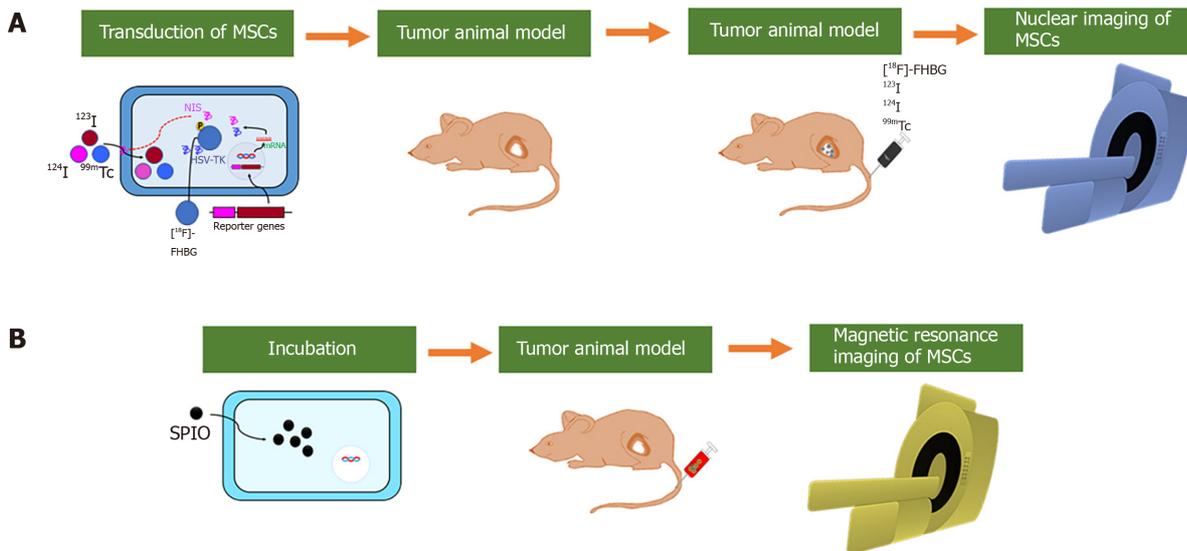


Figure 3 Schematic illustration of the labeling strategy for *in vivo* tracking of mesenchymal stem cells by nuclear and magnetic resonance imaging. A: Gene transduction of sodium iodide symporter (NIS) or herpes simplex virus-thymidine kinase (HSV-TK) into mesenchymal stem cells (MSCs) can aid radiotracers (^{123}I , ^{124}I and $^{99\text{m}}\text{Tc}$) in entering MSCs. MSCs-NIS are injected into tumor-bearing mice followed by the injection of radiotracers. *In vivo* nuclear imaging (positron-emission tomography, camera imaging, and single-photon emission computed tomography) can visualize migration of the MSCs; B: MSCs can be incubated with molecules including small superparamagnetic iron oxide (SPIO) or SPIO coated with gold-nanoparticles (SPIO@Au-NPs). SPIO-labeled MSCs are injected into tumor-bearing mice, and *in vivo* magnetic resonance imaging can visualize migration of the MSCs. MSCs: Mesenchymal stem cells; NIS: Sodium iodide symporter; HSV-TK: Herpes simplex virus-thymidine kinase; $^{99\text{m}}\text{Tc}$: Technetium-99m; ^{18}F -FHBG: 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl) guanine; SPIO: Superparamagnetic iron oxide.

combination with bioluminescence imaging (BLI) are widely utilized for the tracking of stem cell viability post-transplantation in living subjects. A light is emitted when Fluc interacts with the substrate D-luciferin; it can be captured by a light-sensitive imaging apparatus including BLI. In a myocardial infarction murine model, BLI revealed the death of MSCs at 3 wk post-transplantation, showing the possible association between survival of the cell and cardiac function^[56]. Another BLI study revealed the death of *in vivo* administered MSCs in a mouse model of peripheral vascular disease^[57]. A previous study utilized the BLI approach to evaluate the mitochondrial function of implanted MSCs using Fluc driven by the NQO1 enzyme promoter in a murine myocardial ischemia/reperfusion (IR) model, which revealed an inverse relationship between the promoter activity and mitochondrial function^[58]. Another study performed by Psaltis *et al*^[59] showed higher oxidative stress in MSCs transplanted in a myocardial IR rat model using rat MSCs expressing Fluc driven by the NAD(P)H p67^{phox} promoter, in comparison with the cells in sham controls.

MSCs can also be labeled with nuclear medicine reporter genes and imaged with PET or SPECT. In this approach, a reporter gene encoding a specific protein or enzyme (including herpes simplex virus type 1 thymidine kinase; HSV1-tk) is presented into the cells. MSCs expressing the construct induce a strong signal because of intracellular retention of suitable probes through their phosphorylation by the action of the reporter proteins, whereas normal cells without the construct exhibited a trivial signal because of the minimal phosphorylation of the probes which go in and out of cells, when PET imaging of tumor models was performed^[60]. HSV1-tk, being a viral protein, is immunogenic; therefore, it is the target of the host immune system, leading to further reduction in sensitivity of the reporter protein. Proteins of mammalian origin including dopamine receptor and mitochondrial tk have been developed as nuclear medicine reporter genes to avoid this issue^[61]. Probes [e.g., 3-N-(2'-[^{18}F]-fluoroethyl)-spiperone (^{18}F -FESP)] for a reporter gene of dopamine receptor are supposed to bind specifically to the membrane protein and be captured with PET. The use of mutant dopamine receptor rather than the wild-type protein is suggested for the prevention of the subsequent biological response, but it will still be able to bind to the probe. The drawback of this strategy is that it produces a limited signal because of the interaction of the receptor with only one ligand molecule^[61]. In one study, authors transplanted human MSCs, expressing a mutated version of the dopamine type 2 receptor into athymic rats. PET imaging using ^{18}F -fallypride showed a strong signal *in vivo* up to 7 d post-transplantation^[62]. Aside from the dopamine receptor and HSV-tk1, the sodium-

iodide symporter (NIS) has also been utilized as a nuclear medicine reporter gene to monitor MSC differentiation^[63] and visualize migration of the MSCs to breast cancer^[64]. NIS gene encodes a transmembrane protein, which is responsible for the influx of iodine into the cells. A previous study derived MSCs from the bone marrow of transgenic mice, expressing NIS and tracking their differentiation into cardiomyocytes *in vitro* after all-trans retinoic acid treatment. The study showed an increased uptake of ¹²⁵I by treated cells, showing a higher reporter gene activity than the untreated cells^[63]. Another study assessed the potential of NIS-expressing MSCs to treat breast cancer *in vivo*. SPECT imaging with ^{99m}Tc indicated the localization of the MSCs to the tumor tissue 14 d after implantation of the cells. The reporter gene of NIS can also be utilized as a therapeutic gene. Following the administration of another NIS probe, ¹³¹I, which emits cytotoxic beta rays, the tumor regressed^[64]. Other examples of nuclear medicine reporter genes utilized with PET and SPECT imaging include the neurotensin receptor subtypes, the somatostatin receptor, and cytosine deaminase^[61]. The use of hybrid reporter genes enables *in vivo* imaging of MSCs with multimodal approaches^[65]. MSCs derived from porcine bone marrow were engineered to express a trifusion protein comprising renilla luciferase, red fluorescent protein, and herpes simplex truncated tk through lentivirus-mediated transfection and injected into a myocardial infarction pig model. Cell monitoring with [¹⁸F]-FHBG PET showed the retention of MSCs in the myocardium 10 d post-injection as well as a smaller infarct size in MSC-treated animals than that in control animals^[66].

Advantages and disadvantages of each labeling approach

Direct labeling: Direct labeling techniques are quick and easy to perform. The concentration of labeling agents can be precisely controlled. Despite these advantages, direct labeling with fluorescent probes and subsequent optical imaging do not always yield the best results due to the decline in concentration and signal attenuation in the tissue over time. As a result, optical imaging is not appropriate for tissues that are located deep inside the body and for long-term monitoring of cells. The development of new probes with better penetration ability may settle the issue of tissue depth.

Radionuclide labeling coupled with PET imaging is a highly sensitive technique since it enables the detection of even a small number of MSCs (6250-25000 cells)^[41,67,68]. Even though some cell types are sensitive to radionuclide labeling, MSCs are relatively tolerant to radiation exposure^[68]. There is also an indication that cytotoxicity may be a function of time instead of being dose-dependent. In that case, it is easy to misinterpret the situations where cell viability looks intact immediately following labeling^[69]. Also, it is impossible to acquire key data relating to cell viability and proliferation with direct labeling techniques. The signal can be acquired regardless of whether the cell is dead or alive and even when the label is not correlated with cells, thereby increasing the probability of obtaining false-positive results. Radionuclides, being high-energy particles, are less prone to tissue attenuation and are thus frequently utilized in a clinical setting.

The advantage of MRI is that it allows us to visualize MSC behavior immediately post-transplantation^[70]. An FDA-approved T1 contrast agent such as gadopentetate dimeglumine (Gd-DTPA; Magnevist) was utilized by Liu *et al*^[71] to track MSCs in animal models of hemorrhagic spinal cord injury.

SPIO labeling may impact the proliferation, metabolic activity, viability, and overall morphology of MSCs, limiting their use^[43]. Interestingly, Feridex (SPIO), a commercial paramagnetic material, demonstrated no effect on the physiological properties of MSCs, proposing their possible usefulness in the clinical setting^[72]. Overall, SPIO labeling has low sensitivity and cannot detect the presence of a small number of cells^[68]. Cellular quantification is challenging with SPIO because of the decline in the label concentration and changes in spatial distribution during cell division and migration, respectively.

Other direct labeling agents such as nanoparticles may interfere with the cellular function. In a previous study, gold nanorods were utilized as contrast agents to image MSCs in mice. The results show that even though these agents did not cause any toxicity or impact cell proliferation, the expression of 1 out of 26 cytokines (interleukin-6) was modified^[73]. Similarly, commonly used radioisotopes for nuclear imaging may impact the proliferation of MSCs at high doses, likely because of underlying DNA damage^[74]. The choice of labeling agent should be carefully weighed depending upon the cell type and application of interest.

In conclusion, direct labeling methods might be ideal for short-term monitoring of cells, to ensure that cells are actually being delivered to the tissue of interest. Other labeling approaches should be considered for investigating the functional attributes of stem cells over a longer period of time.

Indirect labeling: Indirect labeling approaches enable long-term monitoring of cell viability through the utilization of constitutive-promoter-driven reporter gene transfection. Under the cytomegalovirus (CMV) promoter, reporter genes are always transcribed and translated into proteins if the cell is viable; however, gene silencing of CMV promoter over time^[75] reduces the signal, thereby leading to diminished production of reporter proteins. This phenomenon could be misinterpreted as a decline in cell viability. Nowadays, constitutive promoters of mammalian origin including ubiquitin and β -actin are being utilized because they are less prone to gene silencing and may be better for determining cell survival. A previous report showed little or no change in phenotype and differentiation capacity of MSCs following the incorporation of reporter genes^[76].

While a reporter protein of green fluorescent protein gives rise to a strong signal, the signal is reduced during tissue penetration due to absorption and refraction, thereby limiting its use to label tissues that are only up to 2 mm deep in the animal body^[77]. Thus, this strategy is more useful for cell sorting during *in vitro* or *ex vivo* analysis of cells.

Reporter genes utilized for PET and SPECT, including HSV-tk1 and NIS, can aid in the identification of live cells *in vivo*. Earlier studies have reported an effective use of NIS for *in vivo* imaging of MSCs in an animal breast cancer model^[64].

Finally, there are concerns that reporter genes may integrate at random sites, possibly resulting in a change of characteristics in the transfected MSCs from their parent cells. Present advances in gene editing technologies have made site-specific DNA integration possible, thereby alleviating the concern^[78]. Many research groups are already developing new probes to meet the criteria set forth by the FDA and other regulatory bodies.

APPLICATION OF NONINVASIVE *IN VIVO* IMAGING OF MSCS IN TREATMENT AND DRUG DELIVERY FOR CANCER

Different attempts have been made to develop cell-based therapies and cell-based drug delivery systems for cancerous diseases^[21,79,80]. Researchers and clinicians are excited about the possibility for MSC-based therapies to treat different tumors and utilize MSCs as a drug delivery vehicle by bioengineering the cells. Recent developments in *in vivo* molecular imaging modalities allow us to understand the fate of MSCs in living subjects. The *in vivo* molecular imaging for visualizing homing of MSCs to target lesions, evaluating their therapeutics effects and proving drug delivery capabilities of MSCs to tumors are discussed with examples below (Table 1).

Optical imaging

Fluorescent imaging: *In vivo* visualization as well as therapeutic effects of tetra-sulfonated aluminum phthalocyanine @ fluorescent nanoparticles-MSC (AIPcS₄@FNPs-MSC) cells have been described previously using human osteosarcoma (Saos-2) tumor-bearing mice. First, the AIPcS₄@FNPs were generated by mixing tetra-sulfonated aluminum phthalocyanine (AIPcS₄; photosensitizer) and poly-methyl methacrylate core-shell fluorescent nanoparticles (FNPs). MSCs were labeled with AIPcS₄@FNPs by incubating them for 1 h in a complete medium. AIPcS₄, AIPcS₄@FNPs, and AIPcS₄@FNPs-MSC were intratumorally injected in tumor-bearing mice, fluorescent imaging was conducted immediately, and AIPcS₄@FNPs-MSC was retained in the tumors following intratumor injection, while AIPcS₄ and AIPcS₄@FNPs migrated to the non-target area of mice. Following imaging, they conducted *in vivo* photodynamic therapy (near-infrared light with an LED source). The concentrated localization of AIPcS₄@FNPs-MSC in the tumor enabled a greater reduction in tumor size following photodynamic therapy than other groups^[81].

Migration and therapeutic effects of MSCs transduced with TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand) (TRAIL-MSCs) were studied in a murine colon cancer (HT29) using macroscopic fluorescence imaging. MSCs were transduced with enhanced green fluorescent protein (EGFP) and TRAIL. PBS, MSC, and TRAIL-MSC were intravenously injected to colon tumor-bearing mice, and fluorescence imaging revealed that TRAIL-MSCs migrated to the tumor at day 10. TRAIL-MSC migration to the tumor impeded tumor growth more than the other groups^[82].

Tumoral migration of MSCs was demonstrated with the use of a NIR lipophilic dye (VivoTrack 680) in a human breast cancer (MDA-MB-231) xenograft mouse model. The MSCs were labeled using the dye, and fluorescence imaging disclosed that

Table 1 Noninvasive *in vivo* imaging of mesenchymal stem cells in treatment and drug delivery for cancer

Imaging	Imaging modality	Labelingmethod/agent	Cell type	Naïve/modified	Cell origin	Subject	Route of injection	Duration	Tumor	Outcome	Clinical translation	Ref.
Optical	Fluorescent	FNPs	BM- MSCs	AIPcS4@FNPs@MSCs	Human	Mice	Intratumor	Immediately	Human osteosarcoma	Inhibition of tumor	Limited	Lenna <i>et al</i> ^[81]
		EGFP	BM- MSCs	TRAIL-MSCs	Mouse	Mice	Intravenous	1-10 d	Mouse colon cancer	Inhibition of tumor	Limited	Zhang <i>et al</i> ^[82]
		VivoTrack 680	BM- MSCs	MSCs	Human	Mice	Intravenous	2-24 h	Breast cancer	Inhibition of tumor metastasis	Limited	Camorani <i>et al</i> ^[83]
	Bioluminescent	Rluc	UC- MSCs	HSV-ttk-MSCs	Human	Mice	Intratumor	1-4 d	Breast cancer	Inhibition of tumor	Limited	Leng <i>et al</i> ^[84]
		Fluc	BM- MSCs	MSC-Tet-TK/ MSC-TK	Mouse	Mice	Intratumor	1 and 5 d	Mouse colon cancer	Inhibition of tumor	Limited	Kalimuthu <i>et al</i> ^[85]
		Fluc	BM- MSCs	MSC-CXCR4	Mouse	Mice	Intravenous	1 and 24 h	Breast cancer	Homing of genetically modified MSCs to tumor	Limited	Kalimuthu <i>et al</i> ^[86]
		Fluc	BM- MSCs	Naïve	Human	Mice	Intravenous	5 to 8 wk	Breast cancer	Homing of MSCs to lung metastatic tumor	Limited	Meleshina <i>et al</i> ^[87]
		Fluc	BM- MSCs	Naïve	Human	Mice	Intravenous	1 and 24 h	Thyroid and breast cancer	In vitro Dox delivery/homing of MSCs to tumor	Limited	Kalimuthu <i>et al</i> ^[88]
		Fluc	BM- MSCs	Naïve	Mouse	Mice	Intravenous	1-11 d	Murine breast cancer	Homing and differentiation of MSCs to tumor	Limited	Wang <i>et al</i> ^[89]
		Fluc	BM- MSCs	MSCs-oncolytic adenovirus	Human	Mice	Intravenous	15 min to 10 d	Murine large cell lung carcinoma	Homing of MSCs to tumor	Limited	Hakkarainen <i>et al</i> ^[90]
		Rluc	BM- MSCs	MSC-e23sFv-Fdt-tBid	Mouse	Mice	Intravenous	24 h	Breast cancer and gastric cancer	Inhibition of tumor	Limited	Cai <i>et al</i> ^[91]
Nuclear	PET	[¹⁸ F]-FHBG	BM- MSCs	MSC-HSV1-TK	Human	Mice	Subcutaneous	4 wk	Murine colon carcinoma	MSCs stably stay in tumor	Yes	Hung <i>et al</i> ^[60]
		¹²⁴ I	BM- MSCs	MSC-hNIS	Human	Mice	Intravenous	72 h	Human hepatocellular carcinoma	Inhibition of tumor	Yes	Knoop <i>et al</i> ^[92]
		¹²⁴ I	BM- MSCs	MSC-hNIS	Mouse	Mice	Intravenous	72 h	Mouse pancreatic tumor	Inhibition of tumor	Yes	Schug <i>et al</i> ^[93]
	γ-camera	¹²³ I	BM- MSCs	MSC-hNIS	Human	Mice	Intravenous	72 h	Human hepatocellular carcinoma	Inhibition of tumor	Yes	Knoop <i>et al</i> ^[92]

		¹²³ I	BM- MSCs	HSP70B-NIS- MSC	Human	Mice	Intravenous	0-72 h	Human hepatocellular carcinoma	Inhibition of tumor	Yes	Tutter <i>et al</i> ^[94]
		¹²³ I	BM- MSCs	MSC-hNIS	Mouse	Mice	Intravenous	72 h	Mouse pancreatic tumor	Inhibition of tumor	Yes	Schug <i>et al</i> ^[93]
		¹²³ I	BM- MSCs	MSC-hNIS	Human	Mice	Intravenous	72 h	Human hepatocellular carcinoma	Inhibition of tumor	Yes	Schug <i>et al</i> ^[95]
		^{99m} Tc	BM- MSCs	MSC-hNIS	Human	Mice	Intravenous	3-14 d	Breast cancer	Inhibition of tumor	Yes	Dwyer <i>et al</i> ^[64]
		^{99m} Tc	BM- MSCs	MSC-hNIS	Human	Mice	Intravenous	3-24 d	Human cervical cancer	Inhibition of tumor	Yes	Belmar-Lopez <i>et al</i> ^[96]
MR	MRI	SPIO	BM- MSCs	MSC-hNIS	Human	Mice	Intravenous	3-24 d	Human cervical cancer	Inhibition of tumor	Yes	Belmar-Lopez <i>et al</i> ^[96]
		SPIO@Au-NPs	BM- MSCs	MSC	Human	Mice	Intravenous	0-72 h	Human glioma	Homing of MSC to tumor	Yes	Qiao <i>et al</i> ^[97]

FLI: Fluorescence imaging; BLI: Bioluminescence imaging; MRI: Magnetic resonance imaging; PET: Positron-emission tomography; γ -camera: Gamma camera imaging; FNPs: Fluorescent nanoparticles; EGFP: Enhanced green fluorescent protein; Fluc: Firefly luciferase; Rluc: Renilla luciferase; SPIO: Superparamagnetic iron oxide; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; HSV1-tk: Herpes simplex virus type 1 thymidine kinase; hNIS: Human sodium iodide symporter; Au-NPs: Gold nanoparticles; HSP70B: Heat-inducible promoter; PDGFR β : Platelet-derived growth factor receptor β ; CXCR4: CXC chemokine receptor type 4.

intravenously injected MSCs migrated to breast cancer and targeting was counteracted by anti-platelet-derived growth factor receptor β (PDGFR β) aptamer (Gint4.T) treatment^[83].

Bioluminescent imaging: A triple fusion gene containing Renilla luciferase (Rluc), red fluorescent protein, and herpes simplex virus truncated thymidine kinase (HSV-ttk) was transduced into human umbilical cord-derived MSCs (Rluc-RFP-HSV-ttk/MSC) and bioluminescent imaging was conducted for visualization of subcutaneously injected Rluc-RFP-HSV-ttk/MSC in mouse models with or without ganciclovir (GCV) treatment. Bioluminescent imaging revealed the death of Rluc-RFP-HSV-ttk/MSC in mice treated with GCV. Moreover, in a breast cancer (MDA-MB-231) xenograft model, Rluc-RFP-HSV-ttk/MSC were injected into the breast cancer xenograft, and GCV treatment led to the death of Rluc-RFP-HSV-ttk/MSC and killed the cancer *via* bystander effects^[84].

Murine MSC migration was observed with the use of Firefly luciferase (Fluc) in a murine colon cancer (CT26) xenograft mouse model. MSCs were transduced with RetroX-TRE (tetracycline response element), expressing a truncated herpes simplex virus thymidine kinase (HSV1-sr39tk) and Fluc2 gene with Tet-On (MSC-Tet-TK/Fluc2) or without (MSC-TK/Fluc2). MSC-Tet-TK/Fluc2 and MSC-TK/Fluc2 cells were intratumorally injected in a mouse colon cancer model. Bioluminescent imaging

revealed that both showed increased cell death in mice treated with GCV in both MSCs with or without Tet-On system. Moreover, in a colon cancer xenograft model, MSC-TK/Fluc2 or MSC-Tet-TK/Fluc2 was co-injected with cancer cells, and GCV treatment led to the mortality of both MSC cancer cells^[85].

Migration of MSCs transduced with or without CXCR4 (CXC chemokine receptor 4) and Fluc2 (MSC-Fluc2 or MSC-CXCR4-Fluc2) toward human breast cancer (MDA-MB-231) was observed with the use of bioluminescent imaging. MSC-Fluc2 and MSC-CXCR4-Fluc2 were intravenously injected to breast cancer-bearing mice, and bioluminescent imaging revealed that MSC-CXCR4-Fluc2 migrated to the tumor at 24 h, but MSC-Fluc2 did not migrate to the tumor. The bioluminescent imaging of the study revealed that the expression of CXCR4 can enhance the migration of MSCs to the tumor *in vivo*^[86].

In vivo homing of human MSCs transduced with luc2 gene (MSCs-luc2) to lung metastasis of human breast cancer (MDA-MB-231) tumor-bearing mice was observed using bioluminescent imaging. MSCs-luc2 was intravenously injected to the tumor-bearing mice and imaged 5 h to 8 wk after administration. Bioluminescent imaging demonstrated that MSCs-luc2 migrated to lung metastasis of human breast cancer in the mice, and the signals were concentrated on the tumor, successfully showing the homing of MSCs to lung metastasis^[87].

A previous study reported *in vivo* homing of human MSCs transduced with Fluc2 using human thyroid cancer (Cal62)- or human breast cancer (MDA-MB-231)-bearing mice. MSC/Fluc2 was intravenously injected into thyroid or breast cancer-bearing mice. Bioluminescent imaging were conducted 1 and 24 h following administration; at 24 h imaging showed that MSC/Fluc2 cells migrated to both thyroid and breast cancer in xenograft mouse models^[88].

Migration of murine MSCs to murine breast cancer (4T1)-bearing mice was observed using bioluminescent imaging. First, the MSCs were transduced with Fluc gene and MSC-Fluc, and breast cancer cells were co-injected intravenously into mice to create a lung metastatic model. In the xenograft model, tumors developed in mice, and then MSC-Fluc was intravenously injected into tumor-bearing mice. Bioluminescent imaging revealed that MSC-Fluc stayed in the lung with tumors cells (1 h and 1, 4, and 6 d) and signals increased after 9 d and 11 d. In the xenograft model, intravenously injected cells migrated to tumors at day 1 and slowly decreased. Then, bioluminescent signals increased from day 8 to day 14^[89].

A previous report described the transduction of Fluc gene and loading of oncolytic adenoviruses (Ad) into human MSCs (Ad-MSC/Fluc). The authors utilized bioluminescent imaging for visualization of subcutaneously injected Ad-MSC/Fluc or MSCs + non-replicating Ad in orthotopic murine lung and breast cancers. Bioluminescent imaging disclosed that the signal from Ad-MSC/Fluc was increased in the lungs at day 3. They concluded that MSCs can be a promising vehicle to deliver oncolytic adenoviruses to the tumors^[90].

In vivo homing of murine MSCs transduced with Rluc and e23sFv-Fdt-tBid (HER2-specific killing by the immunoapoptin, called MSC-RT) was observed in orthotopic breast cancer (4T1) and orthotopic gastric tumors (SGC-7901) mouse models. MSC-RT was intravenously injected into tumor-bearing mice, and bioluminescent imaging at 24 h showed migration of MSC-RT to both tumors, followed by inhibited growth^[91].

Nuclear imaging

PET imaging: PET imaging was utilized to visualize HSV1-tk transduced MSCs (MSC-HSV1-tk) migration to murine colon carcinoma (HT-29Inv2) in a mouse model. MSC-HSV1-tk were injected intravenously into the mice, and PET imaging ¹⁸F-labeled 9-(4-fluoro-3-hydroxymethylbutyl)-guanine ([¹⁸F]-FHBG) revealed migration of the MSCs to the tumor^[60].

In vivo homing and the therapeutic effects of labeled human MSCs transduced with NIS (NIS-MSCs) were demonstrated in hepatocellular carcinoma (Huh7) tumor-bearing mice by PET imaging. NIS-MSCs were intravenously injected into tumor-bearing mice and imaged 72 h following the administration of cells, ¹²⁴I was then injected into the mice. PET imaging showed that NIS-MSCs migrated to the hepatocellular carcinoma. Moreover, they showed a reduction in tumor size and better survival following ¹³¹I therapy^[92].

PET imaging was utilized to visualize migration of mouse MSCs to pancreatic tumor in a mouse model. MSCs were transduced with NIS (NIS-MSCs), and NIS-MSCs or MSCs were injected intravenously to tumor-bearing mice. PET imaging was carried out 3 h after ¹²⁴I injection, and revealed the accumulation of ¹²⁴I in the tumor of NIS-MSC-injected mice, showing NIS-MSC migration to the tumor. Moreover, these findings indicated that ¹³¹I therapy reduced the tumor size and increased survival^[93].

Gamma camera imaging: *In vivo* homing and the therapeutic effects of human MSCs indirectly labeled with sodium-iodide symporter (NIS) (NIS-MSCs) were observed in hepatocellular carcinoma (Huh7) tumor-bearing mice by PET imaging. NIS-MSCs, NIS-MSCs+NaClO₄, and MSCs were intravenously injected into tumor-bearing mice, and ¹²³I was injected into mice at 72 h following the administration of the cells. Gamma imaging showed high signals in mouse tumors injected with NIS-MSCs, but no significant signals in MSCs+NaClO₄ or naïve MSC-injected mice. These findings showed that NIS-MSCs successfully migrated to hepatocellular carcinoma. Moreover, they also showed that ¹³¹I therapy reduced the tumor size and increased survival^[92].

As mentioned earlier, NIS-MSCs or MSCs were intravenously administered to tumor-bearing mice. Gamma camera imaging with ¹²³I revealed tracer accumulation in tumors in NIS-MSCs-injected mice. Moreover, the authors showed that ¹³¹I therapy reduced the tumor size and increased survival^[93].

A heat-inducible HSP70B promoter-driven NIS was transduced in MSCs (HSP70B-NIS-MSCs) and showed the migration of MSCs to a hepatocellular carcinoma (Huh7) xenograft in a mouse model. HSP70B-NIS-MSCs were intravenously injected into mice followed by hyperthermia (41°C) and control (normothermia at 37°C) 3 d later. Gamma camera imaging with ¹²³I revealed the migration of HSP70B-NIS-MSCs to tumors and induction of NIS expression by hyperthermia. Moreover, the hyperthermia and ¹³¹I treatment reduced the tumor size and increased survival^[94].

In vivo homing of human MSCs transduced with NIS gene was observed in hepatocellular carcinoma (Huh7)-bearing mice by gamma camera imaging. NIS-MSCs were intravenously injected 24 h after tumor irradiation (0, 2, or 5 Gy) in an animal model. Gamma camera imaging with ¹²³I revealed increased uptake of ¹²³I by the tumor, which enhanced the migratory capacity of MSCs^[95]. SPECT imaging was previously used to visualize the migration of NIS to transduced MSC (NIS-MSC) breast cancer (MDA-MB-231) in a mouse model. NIS-MSCs were injected intravenously into tumor-bearing mice. For SPECT imaging, ^{99m}Tc was injected into mice 3 d after the injection of cells. SPECT imaging revealed increased uptake of the tracer in tumors of NIS-MSCs injected mice, which shows the localization of MSCs in the tumor and expressed NIS in the MSCs. ¹³¹I therapy resulted in a reduction of tumor size^[60].

In vivo homing of different MSCs acquired from various sources (bone marrow, adipose tissue, epithelial endometrium, stroma endometrium, and amniotic membrane) to cervical cancer (HeLa) was observed in an animal model. The NIS gene was transduced into MSCs (MSC-NIS) and the cells were imaged with SPECT/CT. MSCs were intravenously injected into tumor-bearing mice, and SPECT/CT imaging with ^{99m}Tc was carried out at 3, 10, 17, and 24 d after the injection of MSCs. The study showed the variable migration ability of MSCs due to their origins^[96].

MRI

As mentioned earlier, *in vivo* tumoral homing of MSCs was observed by direct SPIO labeling in mice with cervical cancer (HeLa) by MRI. Following the intravenous injection of SPIO-labeled MSC-MRI, the recruitment of SPIO-labeled MSCs to tumors was observed^[96].

Migration of MSCs to glioma was studied by labeling MSCs with nanoparticles containing SPIO coated with gold (SPIO@Au) and imaging with MRI. SPIO@Au-MSCs were injected *via* the intravenous route to mice bearing brain gliomas. MRI revealed migration of the MSCs to the tumor^[97].

FUTURE OUTLOOKS

While studies in small animals can yield a lot of information related to the behavior of MSCs, it may not be relevant when it comes to delivering the cells to large animals or humans due to differences in their physiology. Since our ultimate goal is to utilize such groundbreaking MSC-based therapies for treating cancerous diseases in humans, a lot of factors including the origins of MSCs, types of reporter genes, routes of administration, and timing of cell delivery will need to be modified depending upon the type of model used. Large animals show the most similarity to humans in terms of size, disease progression, weight, and overall anatomy^[98]. Thus, these animal models are widely utilized to test different imaging and therapeutic modalities.

Tracking of MSCs post-transplantation in large animals is practicable with the use of indirect labeling approaches^[99]. There are some challenges associated with imaging

large animals, including low sensitivity and difficulty in quantifying cells. Also, several factors can impact the survival of transplanted cells including limited cell-to-cell and cell-microenvironment contact, tissue hypoxia, and host immune response. Efforts should be made to increase cell viability at the desired site to acquire an optimal therapeutic benefit while minimizing toxicity.

The adaptation of *in vivo* imaging approaches to track MSCs in clinical trials will need careful assessment of pharmacokinetic information relating to cell therapies. If incorporated at an earlier phase of clinical trials, imaging approaches can give insights into optimal dosage, frequency of administration, and retention time for cells by helping to create dose-response curves. Also, imaging can yield key information relating to the *in vivo* functional properties of administered cells including viability, proliferation, and differentiation. A quick search on the clinicaltrials.gov site shows more than 400 ongoing trials exploring the potential of MSCs to treat different diseases^[100]. It is essential to remember that each patient may respond differently to each therapy. Some patients may clear cells faster than others because of a strong immune reaction, leading to changing responses to certain cell therapies^[101-103].

It is believed that *in vivo* imaging (especially PET/MRI due to their high sensitivity and ability to provide meticulous anatomical information) will serve as a powerful tool to evaluate *in vivo* therapeutic cells in the future. Presently, *in vivo* imaging modalities are utilized solely for short-term monitoring of administered cell survival. There are various regulatory obstacles in achieving long-term cell tracking with reporter genes in humans, the technique that could be useful for understanding the long-term therapeutic effects of MSC-based cancer therapy. One solution could be the utilization of endogenous reporter genes of human origin, which has been previously tested in preclinical models^[104]. Finally, with long-term tracking, it is possible to determine the fate of MSCs and how they contribute to the eradication of cancerous diseases.

CONCLUSION

Recent progress in noninvasive imaging technologies has taken the field of cell therapies to the next level. In this review article, essential developments in labeling technologies, imaging modalities, and how they are able to contribute to the development of MSC-based cancer therapies have been exhaustively described. The exact roles of MSCs in cancerous diseases are unclear because they have been shown to promote and inhibit the growth of different tumors at the preclinical level. Presently, MSCs are being utilized for delivery of therapeutic agents in cancer models, thereby opening new avenues for personalized cancer therapies in the future. To improve the clinical utility of MSC-based therapies, it is crucial for us to understand how MSCs interact with cancer cells *in vivo*. The extracellular vesicles originating from MSCs can also be utilized for cancer treatment to escape safety issues relating to the administration of live MSCs. MSCs have given us a new hope to develop safe and effective intervention strategies against cancer. Combined with long-term monitoring, a lot can be learned regarding the hidden potentials of MSCs and their variable fate in humans.

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