Concise Review: Nanoparticles and Cellular Carriers-Allies in Cancer Imaging and Cellular Gene Therapy?

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Abstract

Ineffective treatment and poor patient management continue to plague the arena of clinical oncology. The crucial issues include inadequate treatment efficacy due to ineffective targeting of cancer deposits, systemic toxicities, suboptimal cancer detection and disease monitoring. This has led to the quest for clinically relevant, innovative multifaceted solutions such as development of targeted and traceable therapies. Mesenchymal stem cells (MSCs) have the intrinsic ability to “home” to growing tumors and are hypoimmunogenic. Therefore, these can be used as (a) “Trojan Horses” to deliver gene therapy directly into the tumors and (b) carriers of nanoparticles to allow cell tracking and simultaneous cancer detection. The camouflage of MSC carriers can potentially tackle the issues of safety, vector, and/or transgene immunogenicity as well as nanoparticle clearance and toxicity. The versatility of the nanotechnology platform could allow cellular tracking using single or multimodal imaging modalities. Toward that end, noninvasive magnetic resonance imaging (MRI) is fast becoming a clinical favorite, though there is scope for improvement in its accuracy and sensitivity. In that, use of superparamagnetic iron-oxide nanoparticles (SPION) as MRI contrast enhancers may be the best option for tracking therapeutic MSC. The prospects and consequences of synergistic approaches using MSC carriers, gene therapy, and SPION in developing cancer diagnostics and therapeutics are discussed.

Disclosure of potential conflicts of interest is found at the end of this article.

Current Issues in Cancer Imaging and Therapy

Approximately 25 million people live with cancer [1] and ~13% of all deaths are attributed to this disease [2] worldwide. As specific molecular technologies improve, cancer is increasingly recognized as a highly heterogeneous disease. Despite improvements in anticancer therapies, the lack of tumor-specificity results in significant treatment-associated morbidity, ultimately limiting efficacy due to dosage limitations. Research priorities must now seek to refine the specificity and accuracy of cancer detection and treatment as well as develop strategies that target a wider repertoire of cancer cells. An important aim should be to achieve optimal patient management and improved quality of life through early detection of cancer and metastases, improved treatment delivery, and monitoring of outcomes through accurate and sensitive imaging techniques. Although magnetic resonance imaging (MRI) and computed tomography (CT) are currently integral to patient assessment and management, lesions <1 cm are still difficult to detect owing to the subjective nature of interpretation that may lead to inaccurate assessment [3, 4].

Recent developments in real-time in vivo imaging technologies using image contrast enhancers offer tangible options to better guide treatment delivery and monitor outcome. Furthermore, improved treatment specificity may be achieved through gene therapy-based approaches. Using viral and nonviral vectors, genetic material can be specifically targeted to cancer cells, for example, to compensate for mutations in tumor suppressor genes, to potentiate anticancer immune responses, or to cause oncolysis [5]. However, obstacles to effective delivery of both contrast agents and gene vectors remain. Immune and reticuloendothelial sequestration or nonspecific vector uptake by nontarget organs dramatically reduces treatment efficacy. No single agent has offered a solution, but recent developments in cancer targeting using stem cell (SC) carriers and nanotechnology have led to innovative

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possibilities. We discuss the prospects of using SCs as gene therapy carriers and review strategies combining these with nanocarriers to facilitate monitoring and therapy.

**SCs as Carriers of Cancer Therapy**

The ability of SCs to migrate to pathological sites including wounds, ischemia, and cancer (including micrometastases) [6–13] underpins their development as carriers of therapy, thus, providing an exciting paradigm for targeted cancer therapeutics. The importance of the microenvironment in tumorigenesis was first recognized in Paget’s seminal (1889) “seed and soil” hypothesis [14]. Stroma provides the architectural framework for tumor development while facilitating molecular crosstalk via cytokines and growth factors to promote cellular turnover and angiogenesis. Thus, tumorigenesis closely resembles wound healing, leading to description of tumors as “wounds that do not heal” [15]. Further, extracellular matrix (ECM) remodeling is mediated by SC and tumor cells [16–18].

SCs from different sources have been explored for biomedical applications: embryonic SC; fetal multipotent SC; induced pluripotent SC; adult multipotent SC comprising neuronal SC (NSC), hematopoietic SC (HSC), and mesenchymal SC (MSC) (reviewed in [11]; Fig. 1 summarizes their properties, potential applications, and drawbacks). Overall, by virtue of their lineage plasticity and tumor tropism, adult SCs display the best attributes for targeting cancer. Both HSC and NSC have been explored with variable success, however, their application is limited either due to issues with production or inadequate characterization (Fig. 1; reviewed in [19–25]).

MSCs and Cancer

MSCs are multipotent stromal cells with the ability to self-renew, differentiate into cells of diverse lineage [27], and migrate to sites of pathology [28]. First isolated as an adherent mononuclear cell fraction of bone marrow (BM) [29], MSCs are present virtually in all postnatal tissues [30]. The following MSCs properties make them ideal therapeutic cellular carriers (Table 1): ease of isolation and expansion in vitro; ease of ex vivo genetic modification; autologous transplantation in patients (overcome issues of host immune responses); and finally, hypoimmunogenicity (suitable for allogeneic transplantsations). Indeed, approval of ~107 clinical trials employing MSCs for regenerative medicine, stroke, and myocardial infarction (http://clinicaltrials.gov/crt2/results?term=Mesenchymal-stem+cells&show_fllds = Y) [47] suggests the clinical feasibility of their use for cancer targeting.

MSCs and Tumor Tropism

MSCs show preferential migration toward sites of inflammation, injury, and cancer [6]. Typically, these are attracted to lesions where they engraft into the stroma and persist: in xenograft experiments, 40% of intratumoral fibroblasts in pancreatic lesions in mice were of BM origin [44]. Although, distributed throughout the tumor mass, both this and subsequent studies have shown a greater concentration of BM-derived cells toward the tumor periphery, indicative of the role of
MSCs in the later stages of stromal induction as regulators of desmoplastic reactions [48]. Thus far, the tropism of MSCs for gliomas [42, 49], pulmonary metastases [50–52], breast cancer metastases [53] ovarian carcinoma [54], and melanoma [55] has been demonstrated in several animal models.

Although not completely understood, MSCs “homing” to cancer may involve recruitment of resident fibroblasts and circulating MSCs into the tumor microenvironment through the release of growth factors and chemokines, where they proliferate and subsequently differentiate into tumor stroma forming fibrocytes, myofibroblasts, and neovascular pericytes [48]. Chemokine-receptor pairs including stromal-derived growth factor SDF-1/CXC chemokine Receptor-4 (CXCR4) [56], monocyte chemotactic protein-1/chemokine (C-C motif) receptor 2 [53], hepatocyte growth factor/c-met [57], and Vascular Endothelial Growth Factor (VEGF)/VEGF receptor [58] together with ECM proteins have been implicated [59] (reviewed in [60]). A clear understanding of these processes is crucial to improve MSCs “homing” to tumors in vivo. Characteristics unique to their migratory phenotype including the chemokine receptor status and triggering events such as cytokine release and matrix metalloproteinase (MMP) production at tumor site need to be identified to determine the optimal biological window for therapeutic MSC targeting of tumors. For example, postresection production of cytokines that recruit MSCs to gliomas [61] could provide a window to target gliomas with therapeutic MSCs to remove residual disease. Further, to achieve optimal tumor targeting, specific identification of nonquiescent SC populations, which can migrate, target, and integrate into tumor tissue is essential. This would require an assessment of relevant receptors on these cells and their responses to biological triggers using molecular imaging and appropriate ex vivo or in vitro three-dimensional models [62].

Overall, the recognition that MSCs “home” toward tumors while evading immune clearance has led to extensive research into their use for cancer-specific gene delivery [11, 48, 50, 55]. A primary consideration for such applications is to ensure their in situ efficacy and survival with the retention of their fundamental properties of migration, differentiation, and hypoimmunogenicity, after modification.

Cancer gene therapy delivered using MSC has been based on suicide-, apoptosis-, anti-angiogenesis-, immuno-stimulatory genes, or oncolytic viral vectors (reviewed in [63]) primarily, using the viral vectors. The use of MSCs as carriers for these vectors [5] can address the drawbacks associated with their direct use including: safety (e.g., insertional mutagenesis when integrating viral vectors [retroviruses] are used) [64]; inadequate tumor targeting; inefficient gene delivery resulting from vector and/or transgene immunogenicity; limited availability of virus-specific “receptors” on cancer cells or inefficient transduction of nondividing cells. Furthermore, ex vivo MSCs manipulation maximize transduction efficiency by allowing for the selection of cells carrying the desired gene before in vivo delivery.

### Viral Vectors and MSC
Transduction of MSCs by integrating retroviral vectors is efficient, but their random genomic integration can lead to unwanted transformation, significantly increasing the risk of secondary malignancies. Despite continuing efforts toward the assessment and accurate mapping of safe insertion sites, currently, the risks may outweigh the advantages. Hence, nonintegrating vectors, such as adenoviruses (Ad), are appealing...
and are the most widely explored for cancer gene therapy, Ad can be grown to high titer (~10^12 virus particles per milliliter), yield high gene expression and importantly, transduce dividing and nondividing cells [64]. However, systemically administered Ad can be rapidly cleared by the immune system and hepatic Kupffer cells [64] and inactivated by Ad-neutralizing antibodies in humans [65]. This substantially compromises the efficiency of Ad gene delivery [66–69]. Because of their hypoinnunogenicity, MSCs may act as a “Trojan Horse” for the delivering Ad-mediated gene therapy directly into tumor lesions. This concept has generated significant interest and is the focus of the following section.

MSCs as Hypoinnunogenic Cellular Vehicles for Adenoviral Vectors

MSCs express major histocompatibility class (MHC)-I antigens, thereby avoiding clearance by natural killer cells, whereas the absence of MHC-II and costimulatory molecules permit immune evasion from CD4+ T-lymphocytes [39]. In vitro studies have demonstrated that MSCs do not cause the proliferation of allogeneic T-cells following interferon (IFN)-γ stimulation [70] and this hypoinnunogenicity persists even after homologous differentiation [40]. Studies in several animal models (rodents, dogs, pigs) have shown that allogeneic-mismatched MSCs can engraft in vivo [71]. Importantly, recent studies have shown that MSC-Ad produce therapeutic transgenes even in the presence of physiological concentrations of sera that would otherwise neutralize adenovirus alone in vitro [52]. Thus, the dual benefits of MSCs homing and their potential for allogeneic transplantation without extensive immune suppression can be exploited to increase Ad-gene delivery specifically to tumor sites.

Efficacy of MSCs Carrying Therapeutic Genes Against Cancer

High metabolic activity of MSC permits high-level transgene expression [72]. The use of MSC carriers to deliver Ad-vectors expressing therapeutic genes has been assessed in several preclinical models of cancer (Table 2). Specific delivery of cytokine transgenes to tumor sites has been attempted to mitigate the toxicities associated with systemic administration of the corresponding recombinant proteins. Unlike systemically administered IFN-β, systemically delivered MSC-expressing IFN-β suppressed tumor growth and prolonged survival in a lung melanoma model [50]. The antitumor effects were attributed to the local production of IFN-β within the tumor, thus, highlighting the importance of MSC engrafment for cancer-targeted delivery [34]. Similar benefits have been achieved using MSC-expressing interleukin-2 [42], fractalkine [74], and tumor necrosis factor-related apoptosis inducing ligand [52] against intracranial glioma, lung metastases, and lung carcinoma, respectively. The localized production of cytotoxic drug metabolites was also achieved using MSC-expressing cytosine-deaminase; local conversion of the prodrg, 5-fluorocytosine to 5-fluorouracil, resulted in inhibition of growth of colorectal cancer [76] and melanoma [77] xenografts.

MSCs have also been used to carry and support the replication of oncolytic viruses, which infect tumor cells when released into the tumor mass. This strategy relies on the optimal balance between minimizing cytotoxicity to the cellular carriers and maximizing cytopathic effects on cancer cells. Thus, systemically delivered MSC-bearing oncolytic viruses have been successful against lung metastases [51] and orthotopic breast and lung tumors [75] displaying cytopathic effects against cancer cells with minimal toxicity to the MSC themselves. Similarly, extended host survival and delayed tumor growth was seen following intraperitoneal delivery of MSC-bearing oncolytic Ad against ovarian cancer [54]. In comparison, the same doses of virus injected systemically showed only liver accumulation [75], validating the MSC cell-carrier approach to more efficiently target cancer. However, the low transduction efficiency of MSC with Ad vectors due to the low expression of Ad-receptor Coxackie-adenovirus receptor (CAR) is a limiting factor [78]. This could not be improved by increasing the multiplicity of infection or time of exposure to the Ad [79]. However, alteration of the Ad tropism with genetic modification of Ad-fiber-knob to contain poly-I-L-lysine or addition of the Ad35 fiber improved MSC transduction by 16–460-fold [80]. Cumulatively, these studies indicate the superiority of Ad-modified MSC over the use of Ad alone, although, their therapeutic success is conditional on the efficiency at which MSC are transduced and their subsequent engrafment and persistence within the tumors.

Limitations of MSC Carriers for Cancer Gene Therapy

Despite tremendous interest in MSC, the unpredictability of their in vivo biological properties such as migration and potential for contribution to the neoplastic phenotype poses a serious obstacle. Some studies have raised concerns that proangiogenic and immunomodulatory properties of MSC may potentiate growth and metastatic capacity of epithelial cancer cells, particularly when MSCs are mixed with the cancer cells prior to implantation [81]. Others have shown no apparent effect of exogenous MSC on tumor progression with proven MSC migration but lack of proliferation and differentiation [82, 83]. Thus, exhaustive investigative studies are essential prior to any clinical application [63], for example, an assessment of the time required to generate pathology-free cells, genetic modification, expansion and phenotypic and genotypic characterization, and then certification for human use needs to be established. The investment in time and resources to produce clinical grade MSC showing acceptable levels of genetic modification and expansion to a therapeutic dose for use in patients is considerable. A timely completion of such characterizations is particularly challenging when dealing with primary cells prior to transplantation. Furthermore, MSC from different sources show different properties [84, 85]. For example, MSC from BM proliferate less efficiently than those from umbilical chord or adipose tissue. Some of these constraints can be addressed by development of immortalized, clonal MSC lines that after exhaustive characterization, especially with respect to their tumorigenicity (e.g., based on type of immortalization gene, its insertion site), may be optimal for clinical use [63].

Additionally, variations in persistence, survival, and interactions of MSC in the tumor microenvironment can affect the duration and level of gene expression at the tumor site [63]. Chemotherapy or the immunogenicity of the transgene and/or vector may impact on MSC survival in situ. Overall, adequately long systemic survival of these carriers is mandated to ensure therapeutic efficacy against cancer. This could in part be supported by the production of immunosuppressive chemokines or cytokines, such as VEGF, Interleukin 10 (IL10), or immunosuppression of T effector-, antigen-presenting- or regulatory T-cells. A better understanding of MSC biology can be exploited to prolong their systemic survival, for example, recognition that CD47 marker expression can prevent SC phagocytosis by macrophages [86, 87]. Ultimately, all new approaches must be assessed using human data to evaluate safety and efficacy. Toward that end, a rapid translation of the findings will be greatly facilitated by

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| Reference | MSC | Vector/transgene | Tumor type/host | Route of MSC administration | Transduction efficiency | Outcomes |
|-----------|-----|------------------|-----------------|-----------------------------|------------------------|----------|
| 50        | hMSC | Ad5 IFN-β       | Xenograft       | S.C admixed A375SM          | MOI = 3,000            | Homing: MSC spread to multiple lung mets, formed capsule after I.V. administration and differentiated to fibroblasts. Tumor: MSC-IFNβ suppressed tumor growth. Duration of inhibition was not permanent. Survival: Increased survival of animals treated systemically with MSC-IFNβ. |
| 55        | hMSC | Ad5 IFN-β       | Xenograft       | I.V. via tail vein           | Not reported           | Homing: not reported (see above). Tumor: MSC-IFNβ treated had lower whole lung weight than control or IFN-β treated. Survival: MSC-IFNβ (L.V.) treated survived longer than untreated controls. S.C. adm had no effect. |
| 41        | hMSC | Ad5 IFN-β       | Xenograft       | I.A carotid (hMSC, mMSC)    | Not reported           | Homing: exclusively to glioma after regional I.A delivery and migrated after I.C. delivery. I.V. (tail vein): failed. Tumor: not reported. Survival: Only MSC-IFNβ delivered regionally (L.T or I.A.) but not distant (S.C. or I.V.) prolonged survival. |
| 73        | rMSC | Modified Ad     | Syngeneic       | I.C admixed with 9L cells    | Not reported           | Homing: rMSCs migrated and infiltrated into tumor bed after I.T injection to glioma, formed capsule-like structure. Tumor: In vivo MRI (Gd-DTPA contrast) MSC-IL2 tumor vol small vs. MSC alone at lethal size at 21 days. Survival: Coinjection of MSC-IL2 + 9L had no significant benefit, but I.T. injection significantly prolonged survival. |
| 74        | mMSC | AdRGD CX3CL1    | Syngeneic       | I.V via tail vein            | MOI = 200 48-hour incubation 80% MSC-AdRGD infected <5% MSC-Ad5 infected | Homing: MSC within lung tumors after I.V. delivery, few in normal lung vs. no homing from fibroblast controls. Tumor: Reduced number of metastatic nodules in MSC-CX3CL1 treated compared with PBS controls. Survival: Treatment with MSC-CX3CL1 significantly prolonged survival. |
| 52        | hMSC | Ad.TRAIL (TNF-related apoptosis inducing ligand) | Xenograft       | L.T.                         | 100 pfu/cell + centrifugation 99% | In vitro coculture of A549 with Ad.TRAIL-MSC increased apoptosis by 27% above background. Ad.TRAIL-MSC effect on A549 in vitro was unaffected by presence of WBC and RBC. In vivo: L.T. injection showed almost complete arrest of tumor growth. |
| Reference | MSC Conditionally replicative Ad | Reference | MSC | Vector/transgene | Tumor type/host | Route of MSC administration | Transduction efficiency | Outcomes |
|-----------|---------------------------------|-----------|-----|------------------|----------------|-----------------------------|------------------------|----------|
| 54        | hMSC Panel included             | 54        | hMSC | Panel included   | Xenograft SKOV3ip1 (human ovarian carcinoma) | LP injection | Ad5/3 limited MSC cytotoxicity but max cytopathic effect on SKOV3 | In vitro: PCR Ad DNA in MSC over time, Ad replicated in MSC carriers dependent on transduction efficiency. Homing: MSC homed preferentially to tumor after LP administration, some trapping in spleen and liver. Tumor: MSC-Ad5/3 treated had delayed tumor growth and lower tumor burden. Survival: MSC-Ad5/3 treatment extended survival. |
|           | Ad5wt                           | 75        | hMSC | Panel included   | Xenograft LNM35 (large cell lung carcinoma) | I.V. via tail vein | Ad5pK7 greater transduction than Ad5RGD | In vitro: Ad targeted to αvβ3 integrins and HSPG had greater MSC infectivity, especially Ad5pK7. Homing: MSC-replication defective Ad luciferase signal in lung whereas MSC-oncolytic Ad Luc signal in Liver. Tumor: not reported. Survival: MSC-oncolytic Ad-treated animals had significant survival advantage over virus only treated. |
|           | Ad5/3                           |           |     |                  |                |                            |                        |                      |
|           | Ad5RGD                          | 51        | hMSC | Conditionally replicative Ad | Xenograft MDA-MB-231 (breast cancer cell line) | LV via tail vein | Ad5/3 limited MSC cytotoxicity but max cytopathic effect on MDA-MB-231 cells | In vitro: hMSC-Ad5/3.CXCR4 increased oncolysis of MDA-MB-231 with time leading to possible viral amplification. Homing: Systemic I.V showed exclusive homing of hMSC-Ad5/3.CXCR4 to lung metastases. Tumor: (lung weight as endpoint) hMSC-Ad5/3.CXCR4 had lower mean lung weight than Ad5/3.CXCR4 or control. Survival: hMSC-Ad5/3.CXCR4-treated animals had greater survival than Ad5/3.CXCR4 or control. |
|           | Ad5RGD.CXCR4                     |           |     |                  |                |                            |                        |                      |
|           | Ad5/3wt                         |           |     |                  |                |                            |                        |                      |
|           | Ad5RGDwt                        |           |     |                  |                |                            |                        |                      |

Ad5/3, Ad5 with chimeric fiber; Ad5+Ad3; Ad5, adenovirus serotype 5; Ad5pK7, Ad5 with integrin-binding RGD motif in its fiber; Ad5wt, Ad5 wild type; AxFAEGFP-F/RGD, adenoviral vector carrying humanized variant of *Aequorea victoria* green fluorescent protein with RGD-mutated fiber under control of a CA promoter; CX3CL1, C-X3-C-motif ligand 1 (Fractalkine); CXCR4, cxc chemokine receptor 4 (Fusin); hMSC, human MSC; I.A., intraarterial; I.C., intracranial injection; IFN-β, Interferon beta; IL-2, interleukin-2; I.P., intraperitoneal injection; I.T., intratumoral injection; I.V., intravenous injection; mMSC, murine MSC; MOI, multiplicity of infection; MSC, mesenchymal stem cells; PBS, phosphate buffered saline; S.C., subcutaneous.
monitoring SC survival and behavior in vivo through use of longitudinal, noninvasive imaging technology [4, 82, 88–91].

MSCs AND IMAGING

To translate MSCs benefits to the clinic, their accurate detection and localization in real time using clinically relevant imaging techniques is essential. An ideal imaging modality should be noninvasive, sensitive, and provide objective information on cell survival, function, and location. In context of cancer, MRI, CT, positron emission tomography (PET), and single photon emission computed tomography (SPECT) are the most explored (specific features of different imaging modalities are reviewed in [3, 91–95]. Overall, while nuclear imaging by PET or SPECT leads to greater sensitivity (>5 × 10^5 cells; [96]), these are primarily limited by lack of anatomical context [97]. MRI provides accurate anatomical detail but does not yield information about cell viability and show poor sensitivity (>10^5 cells; [98]). Although, none of these modalities is ideal, MRI is the most preferred for cellular tracking (comprehensively reviewed in [95, 99–102]). Through detection of proton relaxations in the presence of magnetic field (1.5 Tesla [T]–3 T for clinical imaging), it provides tomographic images with excellent soft tissue contrast and can locate the cells of interest in context of the surrounding milieu (edema or inflammation) [103–105] without the use of harmful ionizing radiations (as with CT, PET, SPECT). In addition, MRI offers a greater tracking window in comparison to PET and SPECT that are limited by the decay of short-lived radioactive isotopes.

During MRI, the intrinsic tissue contrast is affected by local microenvironment including magnetic inhomogeneities of the contrast agents, usually measured as changes in two relaxation time constants T1 (brightening) and T2 (darkening) times [106]. In this context, nanotechnology-based contrast agents have rapidly come to the forefront to improve SC detection in situ. In the following sections, after a brief introduction of the nanotechnology platform as it applies to cancer targeting its potential synergistic applications involving the MSCs carriers are discussed.

Nanotechnology and Cancer: Potential for Synergies with Cellular Carriers for Targeting Cancer

Initiated by the discovery that particles of ~50–100 nm “passively” accumulate in cancer deposits, nanotechnology has fast emerged as a tool for imaging and/or delivery of therapies in oncology. This passive uptake occurs via an “enhanced permeability and retention effect” where inherently leaky tumor vasculature coupled with poor intratumoral lymphatic drainage allows extravasation and entrapment of the nanoparticles [107, 108]. Further, nanoparticle surfaces can be modified with cancer-specific antibodies or peptides for the “active” targeting of tumor cells [109]. Thus, nanotechnology platforms offer flexibility and versatility. Not only can nanoparticles deliver targeted therapeutic payloads (drugs or genes), their intrinsic components can simultaneously serve as enhancers for imaging [110]. For optimal targeting and efficacy in vivo, these particles should be biocompatible (based on their size, shape, surface coatings, and chemical or immunotoxicity [111, 112]), easily targeted (through surface interactions with cancer targeting antibodies, peptides or ligands), and easily tracked by virtue of their composition to allow clinical imaging [110].

Of the ever expanding catalogue of nanoparticles including polymers, dendrimers, liposomes, carbon nanotubes, nanoshells, and magnetic nanoparticles [110], several have gained Food and Drugs (FDA) approval for cancer therapeutics (Doxil, DaunoXome) and imaging (Resovist) [107, 110, 113]. However, their first clinical application may be as imaging agents [114–116] and in that the best developed are superparamagnetic iron-oxide nanoparticles (SPION).

SPION comprise a crystalline iron-oxide core coated with biocompatible materials such as, dextran, starch, or polyl derivatives, that confer stability in vivo and can be conjugated with cancer-targeting ligands or gene-vectors for active targeting. These display magnetism only under the influence of an external magnetic field [117], which also avoids self-aggregation. Importantly, SPION are biocompatible and are eliminated through the body’s normal iron metabolism. SPION have been studied for cancer therapy (hyperthermia), magnetic field-assisted targeting, and as contrast enhancers for MRI and targeted molecular imaging [118, 119]. The promise shown in such studies have initiated clinical evaluation of SPION for the detection and management of liver metastases with enhanced sensitivity of up to 95% [120] to nodal metastases in both head and neck [121] and genitourinary cancers [122, 123]. Although these studies have shown SPION usage to be safe [124], some issues associated with their use need to be addressed.

Issues with Use of Iron-Oxide-Based Nanoparticles.

Major limitations of SPION beyond MRI of the Reticuloendothelial system (RES), include their uptake by phagocytic cells leading to their rapid clearance from the blood [125], in vivo toxicity resulting from the coating materials, and surface chemistry together with unwanted cellular or tissue distribution [118, 126]. SPION can also cross the blood-brain barrier and accumulate in the liver (80%–90%), spleen (5%–8%), and BM (1%–2%) [127]. Their ability to agglomerate in the presence of a magnetic field can cause embolization [126, 128]. Excessive iron-oxide could also lead to an imbalance in its homeostasis and may lead to toxicity [129]. Therefore, toxicity of any new formulations of SPIONs has to be established and would require extensive characterization terms in terms of SPION composition, coatings, size, and dosing regimens in vivo. Thus, the use of the nanoparticles under the “camouflage” of MSCs may resolve some of these issues. Particularly for cellular tracking, the delivery of nanoparticle-labeled MSCs directly into tumor deposits will not only allow the tracking of the labeled cells but also the targeted tumor deposits.

Magnetic Nanoparticles and Tracking of SCs In Vivo

Several paramagnetic and magnetic nanoparticles have been evaluated for labeling SCs to enhance their tracking by MRI. Paramagnetic gadolinium (Gd-) and Mn-based nanoparticles lead to image brightening (T1-based) while those based on SPIONs (50–200 nm), ultraSPION (~35 nm), and micronized (MPION) lead to image darkening (T2 and T2*-based) [114, 130]. Of these, only SPION are approved for clinical imaging and are the general focus of this review.

SPION and MRI of SCs. SPION display greater magnetic susceptibility in comparison to conventional Gd and engender significant signal loss to delineate areas of interest. Thus, SPION-labeled cells display a “blooming” artifact that extends beyond the size of particles, making the cells more visible for detection. Clinically, about 1–30 pg Fe per cell is adequate for detection of labeled cells by MRI without alterations in the proliferation, migration, differentiation, reactive
oxygen species formation, and apoptosis rates [118, 131]. Hence, with the increasing use of MSCs for therapy of tissue injury [132, 133], MRI tracking protocols have gained prominence generating crucial information about their migration and survival. MRI signals from intramyocardial implanted SPION-labeled MSC could be detected for up to 16 weeks (Fig. 2A) [132–134] and specific migration of intravenously given SPION-labeled MSCs to the infarct area and not to the healthy surrounding viable myocardium was shown [136]. Further, through MRI of SPION-labeled porcine MSC, improved survival in the infarct zone than in healthy myocardium was shown [137]. Similarly, in models of brain injury and stroke, injury-specific migration of SPION-labeled MSCs (injected contralateral to the area of injury or infused intravenously) could be tracked by MRI (Fig. 2B) [8, 28, 138, 139]. Thus, MRI-based demonstration of retention of injury-specific migration and improved survival of labeled MSCs at the site of injury suggests the feasibility of this approach in clinical oncology.

**Targeting Cancer and Cellular MRI.** In cancer, to date, cellular MRI has primarily been explored in glioma models with most studies employing EnSC or NSC. MRI of SPION-labeled endothelial progenitor cells demonstrated their tumor tropic migration and differentiation into neovasculature within intracranial glioma (Fig. 2C) [135, 140]. Given their neuronal bias, NSC carriers are the most explored for targeting glioma, (NSC literature for reference: [9, 35, 25, 63, 99, 141–143]). Indeed, through MRI of magnetically labeled NSC, their seeding, migration, homing to invading tumor cells has been evaluated successfully clearly indicating the promise of such combinational approaches [9, 144–151] for tracking cellular carriers to cancer lesions. In that, MSCs have generated recent interest as unlike NSCs, these are readily expandable with minimal...
ethical issues. MRI of intravenously infused SPION-labeled MSCs demonstrated specific migration toward glioma MRI [49] in a temporal-spatial pattern showing initial distribution throughout the tumor with subsequent concentration at the periphery (Fig. 2D) [49]. MRI at 1.5 T could detect the signal for over a week with ensuing decay after 14 days, but could be improved by MRI at higher magnetic field strengths. As glioma has a diffuse distribution and spreads beyond the original site [152], the ability of MSCs to home toward metastatic glioma highlights their potential to “track” the migration of cancer. The translation of such noninvasive imaging techniques toward a broader repertoire of cancers is now being explored.

Approaches to Improve MRI Sensitivity and Duration

Magnetic-nanoparticle-labeled cells face limitations typical of exogenously labeled cells such as the dilution of signal with cell division limits the duration of MRI tracking; attenuation of signal down to 42% of the original after 8 weeks was observed [153]. Further, asymmetric sequestration of label during cell division may compromise detection accuracy [154]. The accuracy of MRI data is also compromised by the inability to distinguish viable and nonviable cells and the generation of false signals from dead cells or those engulfed by macrophages [155]. This has initiated interactive research to improve cellular MRI sensitivity and accuracy; a discussion of some of the approaches follows.

Efficient labeling of MSCs can improve detection by magnifying MRI signals [102, 156]. Given that spontaneous SPION uptake is minimal in virtually all cell types apart from those of the RES [157], attempts have been made to increase iron loading into cells through SPION derivatization with peptides [158], dendrimer coatings [159], combination with transfection agents (TAs) [131, 160–162], and electropropertion [163] with variable success (Table 3). Currently, the most widely accepted protocols involve combining SPION with TAs such as poly-L-lysine and protamine-sulfate, achieving high labeling efficiency approaching 100% [161, 162]. However, the narrow therapeutic index for titration of these TAs raises the chance of cytotoxicity, changes in the gene expression or their migratory ability [168, 180, 181]. Furthermore, while SPION-TA-transduced MSCs display unaltered adipogenic and osteogenic differentiation, there is continued debate regarding their deleterious effects on chondrogenesis [131, 163]. The crucial emphasis on new ways to maximize iron internalization in MSCs while limiting toxicity and impact on normal MSCs properties is needed (Table 3).

Use of Other Nanolabels Can Improve MRI Detection of SCs.

Given that signal gain (T2 contrast) is more specific and easier to interpret than signal loss (T2 contrast), paramagnetic manganese-oxide- or gadolinium-oxide-based nanoparticles (T1 contrast enhancers) may provide an attractive alternative [99, 101, 182]. Gd-oxide nanoparticles have appeal because Gd-chelates are approved for clinical MRI and have been used to trace human NSC or MSC [183]. However, potential mitochondrial toxicity [184, 185] compounded by a requirement of greater molar quantities for optimal imaging has limited the interest in their use for MRI. Novel paramagnetic fluorinated nanoparticles have recently been shown to display high specificity with both clinical and high field MRI. Given the absence of endogenous fluorine (F) in the body, hot spot (19F) MRI images of labeled cells were generated with negligible background. Ahrens et al., tracked 19F-nanoparticle-labeled dendritic cells (using cationic perfluoropolyether) to the regional lymph-node after injections into the foot pad of mice [186]. For a complete picture, though, the hot spot image requires overlaying with a simultaneous proton image (standard 1H MRI) [150, 187] (Fig. 3B). Despite the benefit of quantifying the labeled cells [191], sensitivity is low as the signal comes only from the labeled cells, while the proton signal draws from a much larger pool within the body and hence, is currently the preferred choice.

Other Approaches. One approach is to utilize gene technology to introduce magnetic susceptibility enhancing genes [192], efficient transduction of these cells would be key to success of such an approach. Toward that end, the magnetic properties of virus or plasmid DNA-conjugated SPION have been harnessed through “magnetofection” to provide superior transduction (up to 500-fold increase) of “hard to transduce” cells with shorter incubation periods [193–195]. We have shown that Ad-conjugated SPION and magnetofection markedly improves the transduction of MSCs (low CAR expression) ex vivo while minimizing vector toxicity through vector reduction in vector dose and incubation time (unpublished data).

Additionally, notable success of approaches employing modification of hardware and imaging protocols as well as synergizing different imaging modalities has pioneered new innovations for future research [105, 114, 196–202]. Some of these approaches are summarized in Table 4 and Figure 3.

Thus, recent developments in SC, gene technology, and nanotechnology platforms against cancer have reached a junction where there is enormous potential to synergize their individual advantages to achieve concomitant tumor-targeted therapy and imaging.

**CONCLUSIONS AND PERSPECTIVES**

The potential synergism between MSCs, gene-therapy, and magnetic nanoparticles offers an exciting innovation that may offer cancer patients greater treatment and disease management options and ultimately better quality of life. The advances in nanotechnology may be combined with MSCs to facilitate their tracking and provide accurate details about their location, viability and survival. For effective cellular therapy of cancer, the carriers need to target cancer deposits irrespective of their size and location, should be traceable and should survive long enough to deliver the therapeutic payload. This will require real-time imaging ability with high spatial and temporal resolution as well as stringent target specificity.

Current clinical probes generally cater to a single imaging modality, however, it is now clear that combining the attributes of multiple modalities will be required to provide a comprehensive assessment of events as they occur [114]. Indeed, this concept, now explored by various research groups will soon be a preferred choice for clinical application. Again, the flexibility of nanotechnology platforms may be a great ally in imaging cell-based therapies. For example, the imaging potential of MSCs labeled with magnetic nanoparticles conjugated with radionuclides will allow the combined advantages of short-term PET sensitivity and the long-term signal persistence of MRI. Further, development of multimodal smart nanoparticles that can simultaneously image and treat cancer with real time monitoring of associated events as they occur [114, 188] is now feasible through the versatility of nanotechnology. However, these particles need to be exhaustively assessed for their biocompatibility and intracellular or in vivo toxicity before they achieve widespread applicability in the clinic.

Given a relatively poor understanding and ability to control MSCs in vivo behavior, their application as carriers of
contrast agents may not be safe [227]. This may be resolved to some extent by combining imaging with a backup suicide gene technology to eradicate misbehaving cells. This can be achieved through smart combinations with tools of gene therapy, for example, through introduction of suicide genes with regulatable promoters. For example, the use of Herpes Simplex Virus (HSV)/tk Gene Directed Enzyme Prodrug Therapy (GDEPT) and radioactive substrate \(^{18}\)F-9-(4-[\(^{18}\)F]Fluoro-3-Hydroxymethylbutyl) Guanine (\(^{18}\)F-FHBG) has been successful in both human and animal studies for PET imaging [228]; the presence of HSV/tk suicide gene can serve as an additional control to eliminate the transduced cell by treatment with the prodrug (Ganciclovir and Acyclovir) which is then converted to a toxic drug by tk. Thus, magnetically labeled MSCs with HSV/tk GDEPT would allow MRI-PET along with the control of cell survival as needed. Both nanoparticles and MSCs can carry gene vectors, hence, there is scope for endogenous expression of reporter genes under tissue or lineage specific promoters [229] or expression of magnetic susceptibility enhancing genes to enhance the accuracy of the imaging data. Such adjuncts may allow additional assessment of cell viability, survival and fate. Use of reporter genes green fluorescent protein or luciferase [192] regulated by lineage-specific promoters may help detect SCs following differentiation, for example, use of cardiac-specific \(\alpha\)-myosin heavy chain promoter to detect SC conversion to cardiac myocytes [230, 231], Tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE) promoter [229] to detect endothelial differentiation, and osteopontin or osteocalcin promoters to detect osteogenic changes [232].

| Strategies\(^1\) | Magnetic nanoparticle type and concentration | Iron loading (µg/cell) | Issues |
|-----------------|---------------------------------------------|-----------------------|--------|
| Linking to HIV-Tat peptide | HIV-Tat peptide contains membrane translocating signal, transports SPION into cells. | | |
| Labeled cell types: | Neural progenitor cells, hematopoietic CD34\(^+\) cells | | |
| Linking to mAbs | Anti-Tfr mAb linked to SPION | | |
| Anti-Tfr mAb binds to Tfr and internalize through receptor-mediated endocytosis | Labeled cell types: | Neural progenitor cells | |
| | | | |
| Magnetodendrimers | SPION coated with carboxylated dendrimer | Carboxylated dendrimer coating SPION core 1–25 µg Fe/ml | 8.5–13.6 | Nonspecific uptake and large surface area of highly charged polymers pose safety concerns [159] |
| SPION coated with carboxylated dendrimer | Charged coating induce membrane bending and internalization | HeLa, NIH3T3 fibroblast, human neural stem cells, human MSCs | |
| SPION-TA | Various TA used | Labeled cell types: | |
| Lipofectamine [168] | Labeled cell types: | Mesenchymal stem cells, human cervical carcinoma (HeLa), lymphoblasts (LADMAC), neural stem cells | |
| Protamine sulfate [131, 169] | | | |
| SPION | Feridexc \(^a\) | 25–50 µg Fe/ml | 1.47–120 | Up to 40% decrease in viability observed at high dose of PLL [168] SPION-PLL may inhibit chondrogenesis of MSC [170] |
| SPION-PLL | Various TA used | Labeled cell types: | |
| Lipofectamine [168] | Labeled cell types: | Mesenchymal stem cells, human cervical carcinoma (HeLa), lymphoblasts (LADMAC), neural stem cells | |
| PLL [168] | | | |
| Proteamine sulfate [131, 169] | | | |
| Protamine sulfate [131, 169] | | | |
| SPION | Magnetoelectroporation | Rapid low-voltage pulses to induce SPION entry | SPION 250–2,000 µg Fe/ml | 2.5–10 | Relatively high iron concentration required to achieve optimal SPION uptake [163] May lead to the formation of hydrophilic pores between cytoplasm and extracellular environment [163] High loss of viability if pulse conditions are not well controlled [171] |
| Labeled cell types: | Rat and human MSC, mouse NSC | | | |

\(^a\)Additional references for readers interested in stem cell labeling: [172–179].
\(^b\)Cross-linked iron oxide nanoparticles.
\(^c\)Feridex, Food and Drug Administration (FDA) approved SPION coated in dextran but recently discontinued.

Abbreviations: HIV, human immunodeficiency virus; LADMAC, mAbs, monoclonal antibodies; MSC, mesenchymal stem cell; NIH3T3, NSC, neuronal stem cell; PLL, poly-L-lysine; TA, transfection agent; SPION, superparamagnetic iron oxide nanoparticles; Tfr, transferrin receptor.
cord blood [233, 234] and show similar tumor homing and functional capacity as BM-derived MSCs. The wide availability of MSCs from these sources and development of well-characterized immortalized clonal stem cell lines may also ease the practical application of MSCs in the clinic. In particular, the potential for MSCs to be transplanted across MHC barriers in humans [71] can be further explored to facilitate ease of donation in future clinical contexts. It must be noted that the specificity of this system is highly conditional on the tumor homing abilities of MSCs and their in vivo behavior, making this a priority research area. Enhanced insight into the mediators of homing will allow for active targeting of tumors by inducing MSCs to overexpress target receptors for homing.

Figure 3. Approaches to improve stem cell tracking by MRI. (A): PET-MRI dual modality imaging using multimodal nanoparticles: (magnetic nanoparticles + radionuclide, 124I), brachial (3 mm; A’, B’, C’) and axillary lymph nodes (D’, E’, F’) could be detected by superimposition (C’, F’) of anatomical MRI images (A’, D’) with the intense red signal images obtained with PET (B’, E’). Reproduced from [188], with permission from ©Wiley-VCH Verlag GmbH & Co. KGaA. (B): 19F Rapid imaging of labeled mononuclear cells (human umbilical cord blood) at both research (11.7 Tesla) and clinical field (1.5 Tesla) strengths: Using multiple perfluorocarbon nanoparticles, green (PFOB) or red (CE), hot spot 19F images (B’: PFOB) and (C’: CE) were generated and could be superimposed with 1H MRI (11.7 T) for anatomical localizations to the mouse legs (D’). Similar results were obtained using 1.5 T MR (E’: 19F image and F’: superimposed 19F and 1H image). The authors were able to detect as few as 2,000 CE-labeled and 10,000 PFOB-labeled cells with 19F MR spectroscopy and 6,000 CE-labeled cells with 19F MRI in vitro. Reproduced from [187], with permission from FEDN of AM Societies for Experimental Bio (FASEB) Journal via copyright clearance center, © 2006 by FASEB. (C): Detection of Ultra Small Superparamagnetic Iron Oxide Nanoparticles (USPION)-labeled cells using fast imaging employing steady state acquisition pulse sequence on a 1.5 T clinical MRI scanner. (A’): Single USPION-labeled cells could be detected using a custom built gradient RF coil and optimized pulse technology. (a): fluorescent image of Dil/superparamagnetic iron-oxide nanoparticles (SPION)-labeled cells localized between two layers of gelatin in an ELISA well, (b) MR image, (c) fusion image, (d) Axial MR showing the localization of cells in a plane. Reprinted from [189], with permission from ©2003Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc. (B’): In vivo MR images detecting SPION-labeled macrophages (signal voids shown by arrows) injected into the mouse brain frontal cortex (a) and cerebellum (b). (c, d) represent the corresponding images of a control mouse. Reproduced from [190], with permission from ©2006 Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Abbreviations: CE, perfluorocrown-5 ether; MRI, magnetic resonance imaging; PET, positron emission tomography; PFC, Perfluorocarbon; PFOB, perfluorooctylbromide.
direct cells to CXCR4/SDF1 axis to facilitate MSCs tumor tropism [59]. Recent evidence of increased MSCs engraftment in tumors following irradiation (releases chemotactic signals) [235, 236] also indicates the potential of using MSC-based gene therapy as an adjuvant following radiotherapy to maximize the removal of residual disease. Finally, highly specific delivery and individualized therapy may be achieved by the choice of therapeutic genes and manipulation of the vectors depending on the cancer type and degree of aggressive therapy required.

Overall, it is clear that there is no single magic bullet to overcome the complexity and heterogeneity of cancer. Multifaceted approaches that exploit the best attributes of MSC biology, nanotechnology, gene-technology, and gene therapy have the potential to overcome hurdles encountered when each is used alone. However, the possibility that such multidimensional modifications may also enhance the danger of unwanted changes in MSCs functional phenotype such as gain of tumorigenic potential or loss of specific migration, cannot be ignored. A rigorous characterization of modified cells with focus toward addressing the potential regulatory issues would be crucial to achieve their speedy translation to the clinic.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.
Combining Nanoparticles & Stem Cells Versus Cancer

REFERENCES

1. Kamargi F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 2006;24:2137–2150.

2. WHO. Available at http://www.who.int/mediacentre/factsheets/fs297/en/index.html. Accessed July 2008.

3. Weissler R. Molecular imaging in cancer. Science 2006;312:1168–1171.

4. Nakashima J, Tanimoto A, Kikuchi E et al. Clinical implications of tumor size and local extent of primary prostatic lesions in prostate cancer patients with metastases: Value of endorectal magnetic resonance imaging in patients with metastases. Urology 2007;70:86–90.

5. Majhen D Ambriovic-Ristov A. Adenoviral vectors—How to use them in cancer gene therapy? Virus Res 2006;119:121–133.

6. Chamberlain G, Fox J, Ashton B et al. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007;25:2739–2749.

7. Sykova E, Jendelova P. Magnetic resonance tracking of implanted adult human mesenchymal stem cells derived from adult marrow. Bone Marrow Transplant 2008;16:10–16.

8. Mabry KS, Brown A, Ranov NG et al. Neural stem cells display extensive tropism for pathology in adult brain: Evidence from intracranial gliomas. Proc Natl Acad Sci USA 2000;97:12846–12851.

9. Tabatabai G, Bahr O, Mohle R et al. Lessons from the bone marrow: How malignant glioma cells attract adult hematopoietic progenitor cells. Brain 2005;128:2200–2211.

10. Corsten MF, Shah K. Therapeutic stem-cells for cancer treatment: Hopes and hurdles in tactical warfare. Lancet Oncol 2008;9:376–384.

11. Hoehn M, Kustermann E, Blunk J et al. Monitoring of implanted stem cell migration in vivo: A highly resolved in vivo magnetic resonance imaging experiment of experimental stroke in rat. Proc Natl Acad Sci USA 2002;99:16267–16272.

12. Ittrich H, Lange C, Togel F et al. In vivo magnetic resonance imaging of iron oxide-labeled, arterially-injected mesenchymal stem cells in kidneys of rats with acute ischemic kidney injury: Detection and monitoring at 3T. J Magn Reson Imaging 2007;25:1179–1191.

13. Fidler IJ. The pathogenesis of cancer metastasis: The 'seed and soil' hypothesis revisited. Nat Rev Cancer 2003;3:453–458.

14. Dvorak HF. Tumors: Wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650–1659.

15. Li H, Fan X, Houghton J. Tumor microenvironment: The role of the stroma in cancer. J Cell Biochem 2007;101:805–813.

16. Chantren CF, Henriot P, Jodele S et al. Mechanisms of pericyte recruitment in tumour angiogenesis: A new role for metalloproteinases. Eur J Cancer 2006;42:310–319.

17. Ottman A, lngenhagen M. Cancer-associated fibroblasts and tumor growth—Bystanders turning into key players. Curr Opin Genet Dev 2009;19:67–73.

18. Oh MC, Lim DA. Novel treatment strategies for malignant gliomas using neural stem cells. Neurotherapeutics 2006;3:458–464.

19. Zhang ZG, Chopp M. Neurorestorative therapies for stroke: Underlying mechanisms and translation to the clinic. Lancet Neurol 2009;8:491–500.

20. Khanna A, Shin S, Rao MS. Stem cells for the treatment of neurological disorders. CNS Neurol Disord Drug Target 2008;7:98–109.

21. Konopleva M, Tabe Y, Zeng Z et al. Therapeutic targeting of microenvironmental interactions in leukemia: Mechanisms and approaches. Bone Marrow Transplant 2009;42:103–117.

22. Markowicz S. Harnessing stem cells and dendritic cells for novel therapies. Acta Polym Pharm 2008;65:625–632.

23. Bielli A, Cesani M. Human hematopoietic stem cells in gene therapy: Pre-clinical and clinical issues. Curr Gene Ther 2008;8:135–146.

24. Altaner C. Glioblastoma and stem cells. N Engl J Med 1986;315:1650–1659.

25. Dwyer RM, Potter-Beirne SM, Harrington KA et al. Monocyte chemokine as a genetic platform for systemic delivery of therapeutic proteins in vivo. Human factor IX model. J Gene Med 2003;5:117–125.

26. Brooke G, Cook M, Blair C et al. Therapeutic applications of mesenchymal stromal cells. Semin Cell Dev Biol 2007;18:846–858.

27. Hall B, Dembinski J, Sasser AK et al. Mesenchymal stem cells in cancer: Tumor-associated fibroblasts and cell-based delivery vehicles. J Natl Hematol 2007;86:8–16.

28. Wu X, Hu J, Zhou L et al. In vivo tracking of superparamagnetic iron oxide nanoparticle-labeled mesenchymal stem cell tropism to malignant gliomas using magnetic resonance imaging. Laboratory investigation. J Neurosurg 2008;109:320–329.

29. Studeny M, Marini FC, Champlin RE et al. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. Cancer Res 2002;62:3603–3608.

30. Stoff-Khalili MA, Rivera AA, Mathis JM et al. Mesenchymal stem cells as a vehicle for targeted delivery of CRAds to lung metastases of breast carcinoma. Breast Cancer Res Treat 2007;105:157–167.

31. Mohr A, Lyons M, Deedigan L et al. Mesenchymal Stem Cells Expressing TRAIL lead to tumour growth inhibition in an experimental lung cancer model. J Cell Mol Med 2008;12:2628–2643.

32. Dwyer RM, Potter-Beirne SM, Harrington KA et al. Monocyte chemokine protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. Clin Cancer Res 2007;13:5020–5027.

33. Konarova S, Kawakami Y, Stoff-Khalili MA et al. Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. Mol Cancer Ther 2006;5:755–766.

34. Studeny M, Marini FC, Dembinski JL et al. Mesenchymal stem cells: Potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. J Natl Cancer Inst 2004;96:1593–1603.

35. Menon LG, Piccinich S, Koniur R et al. Differential gene expression associated with migration of mesenchymal stem cells to conditioned medium from tumor cells or bone marrow cells. Stem Cells 2007;25:520–528.

36. Forte G, Minieri M, Cossa P et al. Hepatocyte growth factor effects on mesenchymal stem cells: Proliferation, migration, and differentiation. Stem Cells 2006;24:23–33.
118 Shubaye V, Pisanic TR, II, Jin S. Magnetic nanoparticles for ther-

diagnostic and drug Deliv Rev 2009;61:467–477.

119 Gao J, Gu H, Xu B. Multifunctional magnetic nanoparticles: Design, syn-

thesis, and biomedical applications. Acc Chem Res 2009;42:1097–1107.

120 Senetrete E, Taourel P, Bouvier Y et al. Detection of hepatic metas-

tases: Ferumoxides-enhanced MR imaging versus unenhanced MR

imaging and CT during arterial portography. Radiology 1996;200:

785–792.

121 Mack MG, Balzer JO, Straub R et al. Superparamagnetic iron oxide-

enhanced MR imaging of head and neck lymph nodes. Radiology

2002;222:239–244.

122 Harisinghani MG, Barentsz JO, Hahn PF et al. MR lymphangiogra-

phy for detection of minimal nodal disease in patients with prostate

cancer. Radiology 2002;226:312–313.

123 Feldman AS, McDougal WS, Martin-Rendon E et al. Iron particles for non-

medical applications. Possibilities and limitations of a new drug delivery system. J Magn Magn Mater 2005;293:483–496.

124 Gupta AK, Gupta M. Cytotoxicity suppression and cellular uptake

specificity to brain tumors. Gene Ther 2004;11:811–818.

125 Shah K. Imaging neural stem cell fate in mouse model of glioma. Nat

Protoc Stem Cell Biol 2009;Chapter 5:Unit 5A 1.

126 Walczak P, Kedziorek DA, Gilad AA et al. Applicability and limita-

tions of MR tracking of neural stem cells with asymmetric cell divi-

sion and rapid turnover: The case of the shiverer dysmyelinated F344 rats. Eur Radiol 2005;15:375–386.

127 Kustermann E, Himmelreich U, Kandal K et al. Efficient stem cell

labeling. Regen Med 2008;3:807–816.

128 Leunger T, Schöpf B, Hofmann H et al. Superparamagnetic nano-

particles for biomedical applications: Possibilities and limitations of

a new drug delivery system. J Magn Magn Mater 2005;293:483–496.

129 Gupta AK, Gupta M. Cytotoxicity suppression and cellular uptake

specificity to brain tumors. Gene Ther 2004;11:811–818.

130 Schachinger V, Erbs S, Barenz RJ et al. Intracoronary bone marrow-

transplantation of allogeneic mesenchymal stem cells inhibits chondrogenesis but not adipogenesis. NMR Biomed 2004;17:553–559.

131 Sadek H, Latif S, Collins R et al. Use of ferumoxides for stem cell

tracking of transplanted bone marrow and embryonic stem cells la-

beled by iron oxide nanoparticles in rat brain and spinal cord. J Neu-

rosci Res 2004;76:232–243.

132 Gupta AK, Gupta M. Cytotoxicity suppression and cellular uptake

specificity to brain tumors. Gene Ther 2004;11:811–818.

133 Seneterre E, Taourel P, Bouvier Y et al. Detection of hepatic metas-
tases: Ferumoxides-enhanced MR imaging versus unenhanced MR

imaging and CT during arterial portography. Radiology 1996;200:

785–792.

134 Stuckey DJ, Carr CA, Martin-Rendon E et al. Iron particles for non-

medical applications. Possibilities and limitations of a new drug delivery system. J Magn Magn Mater 2005;293:483–496.

135 Gupta AK, Gupta M. Cytotoxicity suppression and cellular uptake

specificity to brain tumors. Gene Ther 2004;11:811–818.

136 Shah K. Imaging neural stem cell fate in mouse model of glioma. Nat

Protoc Stem Cell Biol 2009;Chapter 5:Unit 5A 1.

137 Auer T, Mikkelsen R, Christensen EE et al. Magnetoelectroportation

and isolation of viable engrafted donor cells from the heart. Stem

Cells 2006;24:1968–1975.

138 Anderson SA, Glod J, Arbab J et al. Noninvasive MR imaging of

magnetically labeled stem cells to directly identify neovascularization in glioma model. Blood 2005;105:2497–2503.

139 Janssens S, Dubois C, Bogaert J et al. Autologous bone marrow-

derived cell transfer in patients with ST-segment elevation myocardial infarction: Double-blind, randomised controlled trial. Lancet 2006;367:113–121.

140 Sykova E, Judelova P, Havyrek V et al. Magnetic resonance tracking of

transplanted bone marrow and embryonic stem cells labeled by iron oxide nanoparticles in rat brain and spinal cord. J Neu-

rosci Res 2005;76:232–243.

141 Brekke C, Williams SC, Price J et al. Cellular multiparametric MRI

of neural stem cell therapy in a rat glioma model. Neuroimage 2007;

37:769–782.

142 Song M, Kim Y, Kim Y et al. MRI tracking of intravenously trans-

planted human neural stem cells in rat focal ischaemia model. Neuro-

sci Res 2009;64:235–239.

143 Ruiz-Cabello J, Walczak P, Kedziorek DA et al. In vivo “hot spot”

MRI imaging of neural progenitor cell fate in rodents. Mol Imaging 2008;7:77–91.

144 Arbab AS, Bashaw LA, Miller BR et al. Intracytoplasmic tagging of

cells with ferumoxides and transfection agent for cellular magnetic

resonance imaging after cell transplantation: Methods and techniques. Transplantation 2005;76:1123–1130.

145 Arbab AS, Yocum GT, Kalish H et al. Efficient magnetic cell label-

ing with protamine sulfate complexed to ferumoxides for cellular MRI. Blood 2004;104:1125–1129.

146 Shah K. Imaging neural stem cell fate in mouse model of glioma. Nat

Protoc Stem Cell Biol 2009;Chapter 5:Unit 5A 1.

147 Waerzeggers Y, Klein M, Miletic H et al. Multimodal imaging of

neural progenitor cell fate in rodents. Mol Imaging 2008;7:77–91.

148 Matuszewski L, Persigel T, Wall A et al. Cell tagging with clini-

cally approved iron oxides: Feasibility and effect of lipofection, particle size, and surface coating on labeling efficiency. Radiology 2005;235:155–161.

149 Lewin M, Carlesso N, Tung CH et al. Tat peptide-derivatized mag-
netic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat Biotechnol 2000;18:410–414.

150 Bulte JW, Douglas T, Witwer B et al. Magnetodendrimers allow

edosomal magnetic labeling and in vivo tracking of stem cells. Nat

Biotechnol 2001;19:1141–1147.

151 Arbab AS, Bawash LA, Miller BR et al. Intracytoplasmic tagging of

cells with ferumoxides and transfection agent for cellular magnetic

resonance imaging after cell transplantation: Methods and techniques. Transplantation 2005;76:1123–1130.

152 Walczak P, Kedziorek DA, Gilad AA et al. Intracellular labeling of

stem cells using magnetoelectroporation. Magn Reson Med 2005;54:

769–774.

153 Bulte JW, Arbab AS, Douglas T et al. Preparation of genetically

labeled cells for cell tracking by magnetic resonance imaging. Meth-

ods Enzymol 2004;386:275–299.

154 Daniels TR, Delgado T, Rodriguez JA et al. The transferrin receptor

part II: Targeted delivery of therapeutic agents into cancer cells. Clin

Cancer Res 2003;9:3910–3917.

155 Trofimenkova U, Himmelreich U, Kandal K et al. Efficient stem cell

labeling for MRI studies. Contrast Media Mol Imaging 2006;5:

27–37.
Montet-Abou K, Montet X, Weissleder R et al. Cell internalization of magnetic nanoparticles using transfection agents. Mol Imaging 2007;6:1–9.

Anderson CJ, Bulte JW, Chen K et al. Design of targeted cardiovascular molecular imaging probes. J Nucl Med 2010;51(suppl 1):155–178. Epub 2010 Apr 15.

Song M, Moon WK, Kim Y et al. Labeling efficacy of superparamagnetic iron oxide nanoparticles to human neural stem cells: Comparison of ferumoxides, monocrystalline iron oxide, cross-linked iron oxide (CLIO)-NH2 and tat-CLIO. Korean J Radiol 2007;8:365–371.

Bulte JW, Ben-Hur T, Miller BR et al. MR microscopy of magnetically labeled neurospheres transplanted into the Lewis EAE rat brain. Magn Reson Med 2003;50:201–205.

Ganarre LC, Payon LF, Marti LC et al. In vitro study of CD133 human stem cells labeled with superparamagnetic iron oxide nanoparticles. Nanomedicine 2008;4:330–339.

Bulte JW, Douglas T, Witwer B et al. Monitoring stem cell therapy in vivo using magnetodendrimers as a new class of cellular MR contrast agents. Acad Radiol 2002;9(suppl 2):S332–S335.

Schafer R, Kehlbach R, Muller M et al. Labeling of human mesenchymal stromal cells with superparamagnetic iron oxide leads to a decrease in migration capacity and colony formation ability. Cytotherapy 2009;11:68–78.

Schafer R, Kehlbach R, Wiskirchen J et al. Transferrin receptor upregulation of human mesenchymal stem cells with superparamagnetic iron oxide. Radiology 2007;244:514–523.

Gilad AA, Waczak P, McMahon MT et al. MR tracking of transplanted cells with “positive contrast” using manganese oxide nanoparticles. Magn Reson Med 2008;60:1–7.

Modio M, Mellinow K, Cash D et al. Mapping transplanted stem cell migration after a stroke: A serial, in vivo magnetic resonance imaging study. Neuroimage 2004;21:311–317.

Anderson SA, Lee KK, Frank JA. Gadolinium-fullerol as a paramagnetic contrast agent for cellular imaging. Invest Radiol 2006;41:332–338.

Brekle C, Morgan SC, Lowe AS et al. The in vitro effects of a bi-modal contrast agent on cellular functions and relaxometry. NMR Biomed 2007;20:77–89.

Ahrens ET, Feili-Harrin M, Xu H et al. Receptor-mediated endocytosis of iron-oxide particles provides efficient labeling of dendritic cells for in vivo MR imaging. Magn Reson Med 2003;49:1006–1013.

Partlow KC, Chen J, Brant JA et al. 19F magnetic resonance imaging for stem/progenitor cell tracking with multiple unique perfluorocarbon magnetobeads. FASEB J 2007;21:1647–1654.

Choi JS, Park JC, Nah H et al. A hybrid nanoparticle probe for dual-modality positron emission tomography and magnetic resonance imaging. Angew Chem Int Ed Engl 2008;47:6259–6262.

Foster-Garner H, Heyn C, Alejski A et al. Imaging single mammalian cells with a 1.5 T clinical MRI scanner. Magn Reson Med 2003;49:968–971.

Heyn C, Ronald JA, Mackenzie LT et al. In vivo magnetic resonance imaging of single cells in mouse brain with optical validation. Magn Reson Med 2006;55:23–29.

Srinivas M, Morel PA, Ernst LA et al. Fluorine-19 MRI for visualization and quantification of cell migration in a diabetes model. Magn Reson Med 2007;58:725–734.

Gilad AA, Ziv K, McMahon MT et al. MRI reporter genes. J Nucl Med 2008;49:1905–1908.

Plank C, Schillingler U, Scherer F et al. The magnetofection method: Using magnetic force to enhance gene delivery. Biol Chem 2003;384:737–747.

Scherer F, Anton M, Schillingler U et al. Magnetofection: Enhancing and targeting gene delivery by magnetic force in vitro and in vivo. Gene Ther 2002;9:102–109.

Bhattarai SR, Kim SY, Jang KY et al. N-hexanoyl chitosan-stabilized magnetic nanoparticles: Enhancement of adenoviral-mediated gene expression both in vitro and in vivo rat mesenchymal stem cells with superparamagnetic iron oxide. J Biomed Nanotechnol 2008;4:146–154.

Pichler BJ, Judenhofer MS, Pfannenberg C. Multimodal imaging approaches: PET/CT and PET/MRI. Handb Exp Pharmacol 2008;109–132.

Lee Z, Dennis JE, Gerson SL. Imaging stem cell implant for cellular-based therapies. Exp Biol Med (Maywood) 2008;233:930–940.

Aisen P. Transferrin, the transferrin receptor, and the uptake of iron by cells. Met Ions Biol Syst 1998;35:585–631.

Aisen P. Ferritin receptors and the role of ferritin in iron transport. Target diag Ther 1991;4:339–354.

Batta K, Zok O, Aisen P. Recycling, degradation and sensitivity to the synergistic anion of transferrin in the receptor-independent route of iron uptake by human hepatoma (HuH-7) cells. Int J Biochem Cell Biol 2004;36:340–352.

Bulte JW, Zhang S, van Gelderen P et al. Neuronotransplantation of magnetically labeled oligodendrocyte progenitors: Magnetic resonance tracking of cell migration and myelination. Proc Natl Acad Sci USA 1999;96:15256–15261.

Genove G, DeMarco U, Xu H et al. A new transgene reporter for in vivo magnetic resonance imaging. Nat Med 2005;11:450–454.

Zurikaya O, Chan AW, Hu X. MagA is sufficient for producing magnetic nanoparticles in mammalian cells, making it an MRI reporter. Magn Reson Med 2008;59:1225–1231.

Alikhe H, Stoppler H, Nocken F et al. In vitro MR imaging of regulated gene expression. Radiology 2003;228:488–492.

Ramadan SS, Heyn C, Mackenzie LT et al. Ex-vivo cellular MRI with b-SPP: Quantitative benefits of T1 over 1.5 T. Magn Reson Mater Phys Biol Med 2008;21:251–259.

Heyn C, Ronald JA, Mackenzie LT et al. In vivo magnetic resonance imaging of single cells in mouse brain with optical validation. Magn Reson Med 2006;55:23–29.

Hinds KA, Hill JM, Shapiro EM et al. Highly efficient endosomal labeling of progenitor and stem cells with large magnetic particles allows magnetic resonance imaging of single cells. Blood 2003;102:867–872.

Shapiro EM, Skrtec S, Shaker K et al. MRI detection of single particles for cellular imaging. Proc Natl Acad Sci USA 2004;101:10901–10906.

Kircher MF, Mahmood U, King RS et al. A multimodal nanoparticle for prepregenerative magnetic resonance imaging and intraoperative optical brain tumor delineation. Cancer Res 2003;63:8122–8125.

Josephson L, Kircher MF, Mahmood U et al. Near-infrared fluorescent nanoparticles as combined MR/optical imaging probes. Bioconjug Chem 2002;13:554–560.

Wang S, Jarrett BR, Kauzlarich SM et al. Core/shell quantum dots with high relaxivity and photoluminescence for multimodality imaging. J Am Chem Soc 2007;129:3838–3856.

Huber MM, Staubli BJ, Kustejlo K et al. Fluorescently detectable magnetic resonance imaging agents. Bioconjug Chem 1998;9:242–249.

Zhang Z, Liang K, Bloch S et al. Nonmolecular multimodal fluorescent radioisotope imaging agents. Bioconjug Chem 2005;16:1232–1239.

Zielhuis SW, Seppenwoolde JH, Mateus VA et al. Lanthanide-loaded liposomes for multimodality imaging and therapy. Cancer Radiobiol Radiother 2006;21:522–529.

Hill JM, Dick AJ, Raman VK et al. Serial cardiac magnetic resonance imaging of injected mesenchymal stem cells. Circulation 2003;108:1009–1014.

de Vries BJ, Lesterhuis WJ, Barentsz JO et al. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. Nat Biotechnol 2005;23:1407–1413.

Park KS, Tae J, Choi B et al. Characterization, in vitro cytotoxicity assessment, and in vivo visualization of multimodal, RITC-labeled, silica-coated magnetic nanoparticles for labeling human cord blood-derived mesenchymal stem cells. Nanomedicine 2009.

Jackson J, Chapon C, Jones W et al. In vivo multimodal imaging of stem cell transplantation in a rodent model of Parkinson’s disease. J Neurosci Methods 2009;183:107–117.

Chapon C, Jackson J, Aboagy EO et al. In vivo multimodal imaging study using MRI and PET of stem cell transplantation after myocardial infarction in mice. Mol Imaging Biol 2009;11:31–38.

Zinn KR, Chaudhuri TR, Szafran AA et al. Noninvasive bioluminescence imaging in small animals. ILAR J 2008;49:103–115.

Myhr G. MR guided cancer treatment system for an elevated therapeutic index—A macroscopic approach. Med Hypotheses 2008;70:665–670.

Cao F, Drukker M, Lin S et al. Molecular imaging of embryonic stem cell misbehavior and suicide gene ablation. Cloning Stem Cells 2007;9:107–117.

Cao F, Lin S, Xie X et al. In vivo visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. Circulation 2006;113:1005–1014.

De Palma M, Venneri MA, Roca C et al. Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. Nat Med 2003;9:789–795.
230 Kang JH, Lee DS, Paeng JC et al. Development of a sodium/iodide symporter (NIS)-transgenic mouse for imaging of cardiomyocyte-specific reporter gene expression. J Nucl Med 2005;46:479–483.

231 Choi SC, Shim WJ, Lim DS. Specific monitoring of cardiomyogenic and endothelial differentiation by dual promoter-driven reporter systems in bone marrow mesenchymal stem cells. Biotechnol Lett 2008; 30:835–843.

232 Kumar S, Mahendra G, Ponnazhagan S. Determination of osteoprogenitor-specific promoter activity in mouse mesenchymal stem cells by recombinant adeno-associated virus transduction. Biochim Biophys Acta 2005;1731:95–103.

233 Lee OK, Kuo TK, Chen WM et al. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 2004;103:1669–1675.

234 Kim SM, Lim JY, Park SI et al. Gene therapy using TRAIL-secreting human umbilical cord blood-derived mesenchymal stem cells against intracranial glioma. Cancer Res 2008;68:9614–9623.

235 Klopp AH, Spaeth EL, Dembinski JL et al. Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. Cancer Res 2007;67:11687–11695.

236 Zielske SP, Livant DL, Lawrence TS. Radiation increases invasion of gene-modified mesenchymal stem cells into tumors. Int J Radiat Oncol Biol Phys 2009;75:843–853. Epub 2008 Oct 11.

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