Stem Cell Rev and Rep (2015) 11:688–698
DOI 10.1007/s12015-015-9605-9

Practical Issues with the Use of Stem Cells for Cancer Gene Therapy

Faranak Salman Nouri [1] · Debabrata Banerjee [2,3] · Arash Hatefi [1,2]

Published online: 28 June 2015
© Springer Science+Business Media New York 2015

Abstract Stem cell-based drug delivery for cancer therapy has steadily gained momentum in the past decade as several studies have reported stem cells' inherent tropism towards tumors. Since this science is still in its early stages and there are many factors that could significantly impact tumor tropism of stem cells, some contradictory results have been observed. This review starts by examining a number of proof-of-concept studies that demonstrate the potential application of stem cells in cancer therapy. Studies that illustrate stem cells' tumor tropism and discuss the technical difficulties that could impact the therapeutic outcome are also highlighted. The discussion also emphasizes stem cell imaging/tracking, as it plays a crucial role in performing reliable dose–response studies and evaluating the therapeutic outcome of treatment protocols. In each section, the pros and cons associated with each method are highlighted, limitations are underlined, and potential solutions are discussed. The overall intention is to familiarize the reader with important practical issues related to stem cell cancer tropism and in vivo tracking, underline the shortcomings, and emphasize critical factors that need to be considered for effective translation of this science into the clinic.

✉ Arash Hatefi
ahatefi@pharmacy.rutgers.edu

1 Department of Pharmaceutics, Rutgers, The State University of New Jersey, William Levine Hall, Room 222, 160 Frelinghuysen Road, Piscataway, NJ 08854, USA

2 Rutgers-Cancer Institute of New Jersey, New Brunswick, NJ 08903, USA

3 Department of Pharmacology, Rutgers-Robert Wood Johnson Medical School, New Brunswick, NJ 08901, USA

Keywords Cancer therapy · Enzyme prodrug · Gene therapy · Mesenchymal stem cells · Stem cell imaging · Stem cell tracking · Suicide genes · Tumor tropism · Stem cell-based cancer gene therapy

Introduction

Recent progress in stem cell research has sparked great interest among scientists because these cells are taken from the patient's own body and can act as an easily accessible cell source for cell transplantation in cancer therapies. One of the attractive attributes of the stem cells is their inherent tumor tropism. This characteristic of stem cells could be exploited to develop effective treatments for patients with tumors that are hard to access or treat (e.g., glioblastoma) [1]. For this purpose, stem cells are first genetically modified ex-vivo to stably express a therapeutic molecule, such as a prodrug-converting enzyme, and are then injected back into the body to migrate into tumors. Later, a prodrug is administered systemically which gets converted into its cytotoxic form by the enzyme inside the genetically modified stem cells. This results in the death of the stem cells as well as neighboring cancer cells through a phenomenon known as "bystander effect" [2–4]. For more information on the use of enzyme/prodrug systems, stem cell source, transduction method and the animal models used for preclinical stem cell-based cancer suicide gene therapy, we would like to invite the readers refer to a well-written review article by Amara et al. (2014) [5]. In comparison to some of the current nanotechnology-based targeted drug delivery systems (nanomedicines) that exist for cancer treatment, stem cell-mediated therapies are believed to provide some distinct advantages. To date, numerous nanomedicines such as viruses, liposomes and polymeric nanoparticles have been developed and utilized to target cancer [6–9]. These drug

carriers are known to be able to target tumor cells passively by taking advantage of tumor's leaky vessels to accumulate and then release the cytotoxic drugs in the tumor environment. This mechanism is termed enhanced permeability and retention (EPR) effect [10, 11]. Because of a better understanding of tumor physiology in recent years, we now know that taking advantage of the EPR effect as the primary source for tumor targeting and treatment may not be applicable to all tumors [12]. For example, it is well-understood that the degree of leakiness of blood vessels significantly varies depending on the tumor type and size, which in turn complicates dose–response correlation studies in patients. In contrast to nanomedicines, the extravasation of stem cells to move from circulating blood to the tumor environment is an active process and not EPR dependent [13]. Diapedesis is the combination of several consecutive cell movements that finally results in the escape of stem cells from blood vessel to surrounding tissues [14]. Therefore, the difference in leakiness of the tumors may not significantly influence the efficiency of the treatment. The emergence of stem cell-mediated cancer therapy as an alternative or complementary approach to current cancer therapeutics has sparked great enthusiasm among scientists because it may be used to carry therapeutic agents actively deep inside the tumor hypoxic environment [13].

This review starts by examining a number of proof-of-concept studies that demonstrate the potential application of stem cells in cancer therapy. Then, it highlights the studies that illustrate stem cells' tumor tropism, followed by discussing the reports that provide evidence to argue otherwise. Subsequently, it delineates various imaging methods for stem cell tracking as it is necessary for performing reliable dose–response studies at both preclinical and clinical levels. In each section, the pros and cons associated with each method are highlighted; weaknesses underlined and potential solutions are discussed. The coverage of the literature in this critical review is not encyclopedic; rather, select examples have been chosen to focus on important issues related to stem cell tumor tropism and stem cell in vivo tracking. These two important subject areas are chosen as the focus of this review because the former determines the therapeutic efficacy and the latter is necessary for validation of therapy response. The discussion emphasizes some of the practical issues, problems and unique challenges that are associated with the use of stem cells for cancer therapy.

Stem Cells in Cancer Therapy: Proof-of-Concept

Several groups in the past decade have performed proof-of-concept studies to demonstrate potential use of stem cells in cancer therapy. In these studies, stem cells were first genetically modified to express therapeutic genes such as interferons (INFs) [15–18], interleukins (ILs) [19, 20], tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [21, 22] or suicide genes [23–26]. Then, they were mixed with tumor cells at different ratios in vitro to co-culture or co-inject in vivo and study the impact of genetically modified stem cells on stimulation/inhibition of tumor growth. For example, Studeny et al. (2002), engineered INF-β expressing mesenchymal stem cells (MSC-INFβ) and co-cultured them with A375SM melanoma cells at 1:10, 1:5 and 5:1 ratios to evaluate their cancer cell growth inhibitory effects in vitro [27]. The number of cells was measured after 72 h and the results demonstrated significant decrease in number of co-cultured MSC-INFβ and A375SM cells as compared to the control group. In another approach, Uhl et al. (2005), used thymidine kinase expressing neural stem cells (NSC-TK) and co-cultured with glioma cells at ratios ranging from 1:1 to 1:20 [28]. GCV treatment was started after 24 h and continued for 48 h. The results of this study showed significant levels of toxicity in the co-cultured cells that were treated with GCV as compared to untreated control. Overall, the highest level of toxicity was observed at 1:1 ratio and lowest at 1:20 ratio as expected. Along with these studies, our group has also used various enzyme/prodrug systems to demonstrate the importance of MSC to cancer cell ratio and its impact on cancer cell killing efficiency [29].

While the studies mentioned above demonstrated the potential use of stem cells in cancer therapy in vitro, others evaluated their use in animal models. Benedetti et al. (2000), were among the first groups who examined the tumor inhibitory effects of genetically modified stem cells in animal models [30]. They first transduced neural progenitor cells with IL4 followed by mixing with C6 glioma cells at 10:1 ratio in vitro. Then, the mixture was injected to the left striatum of the Sprague–Dawley rats. The results revealed long term survival of 50 % of rats in co-injection groups as compared to control group that only received C6 glioma cells. Later, Kucerova et al. (2008), performed a similar experiment but with different stem to cancer cell ratios [4]. They first mixed yeast fusion cytosine deaminase:uracil phosphoribosyl transferase expressing mesenchymal stem cells (MSC-yCD:UPRT) with A375 melanoma cells at ratios of 1:10 and 1:5 and then injected into mice. All groups were treated with prodrug 5-fluorocytosine. Although all mice in 1:10 ratio treatment group developed tumors by the study's end point, but the tumor onset was twice longer than the control group. This difference was even more pronounced in treatment group which received stem to cancer cells at ratio of 1:5. At this ratio, 89 % of animals in treatment group were tumor free at the end of the study. Many other groups have used similar approaches to demonstrate the potential application of such genetically modified stem cells in cancer therapy especially in hard-to-reach tumors [31–33]. The ratio of the stem cells to the tumor cells in these studies also proved to be of paramount importance and a determining factor. This ratio could potentially

pose a significant challenge in clinical settings as maintaining the high stem cell to cancer ratio for aggressive tumors may require frequent injection of large doses of MSCs. Since stem cell-based cancer gene therapy has recently reached Phase I clinical trials for evaluation of safety (Clinicaltrials.gov, NCT02015819), it will be very interesting to see how this issue can be addressed in Phase II/III (evaluation of efficacy).

The studies discussed so far point at several successful outcomes, but there are some important issues remained unresolved. For example, no optimum stem cell to cancer cell ratio has been determined that could guarantee successful elimination of tumor cells as such outcome depends on multiple factors including cancer cell type and stage and resistance of cancer cells to the therapeutic molecules. In addition, in most of the studies discussed so far stem cells are co-injected with cancer cells at the time of tumor implantation with almost uniform dispersion throughout the tumor mass. Unfortunately, this is not the case in the clinic as the tumors are formed first in patients and then the stem cells are injected into the body to reach and infiltrate the tumor mass. Another confusing observation that has sparked debate among scientists is the effect of naïve MSCs on tumor growth. While some studies report inhibitory effect of naïve MSCs on tumor growth [34–38], others show stimulatory effects [39–41]. There are also studies that have observed neither inhibitory nor stimulatory effects. Overall, it appears that the timing of MSC introduction into tumor masses may be a critical element [42]. For example, in studies that reported stem cells' inhibitory effect on tumor growth, the direct contact of MSCs with cancer cells during tumor initiation were somewhat obstructed (e.g., implantation into a gelatin matrix or intravenous delivery of MSCs). In contrast, in studies that reported tumor growth promotion, MSCs were mixed with tumor cells and then co-injected to form tumors. The existence of MSCs during early tumor growth may have facilitated angiogenesis which is necessary for tumor initiation. In a recent review by Klopp et al. (2011), these discrepancies are dissected and several important points highlighted [42]. Therefore, we invite the readers to refer to this article for more detailed information.

Tumor Tropism of Stem Cells

Evaluation of Tumor Tropism of Stem Cells in Vitro

Stem cells are derived from different parts in the body such as embryo, fetus, cord blood and adipose tissues among others [43]. Regardless of the source, it is broadly claimed in literature that stem cells possess intrinsic tropism towards tumors. However, it needs to be emphasized that factors such as tumor type and stem cell lineage and size could impact the number of stem cells that reach tumors [44]. This tumor tropism is attributed to many factors including tumor cell-specific receptors and soluble tumor derived factors such as stromal cell-derived factor-1, tumor necrosis factor (TNFα), and interleukins among other inflammatory mediators [45, 46]. The most commonly used test for in vitro demonstration of the tumor tropism of stem cells is migration assay (Fig. 1). Using this assay, many groups have shown that stem cells have preference to migrate toward cancer cells relative to normal cells [47, 48]. Our group has also observed and reported such tropism toward cancer cells in vitro [29]. Although informative, but migration assay may not be a perfect experiment to prove tumor tropism of the stem cells because it is extremely difficult to mimic the in vivo conditions and include all the factors which may alter the fate of the stem cells in the body. Therefore, more studies at the in vivo level are required.

Evaluation of Tumor Tropism of Stem Cells After Injection in Tumor Vicinity

In the past decade, tumor tropism of the stem cells has been studied in animal models after local injection of stem cells in close proximity of tumors. One very well-studied cancer model using this approach is glioma where stem cells are injected intracranially in the contralateral hemisphere relative to tumor site followed by evaluation of their migration toward tumors [49–51]. In majority of the studies, it has been observed that stem cells migrated from the injection site to contralateral hemisphere and successfully reached the tumors [52–54]. Similar observations have also been reported with tumors of peritoneal cavity such as ovarian. For example, Kidd et al. (2009), used HEY cell line to induce intraperitoneal (IP) ovarian tumors in SCID mice [55]. Two weeks later, luciferase expressing MSCs were injected IP into tumor-bearing and tumor-free (control) mice. Live animal imaging was performed on days 1, 7 and 14 to track stem cells' migration. In both cancerous and normal mice, stem cells dispersed initially in peritoneal cavity but after 14 days the signal was only detectable in tumor bearing mice. In this study, the presence of the stem cells in tumors was confirmed by detecting luciferase signal followed by immuno-histochemistry assays after euthanizing the animals and dissecting the tumor and other organs (Fig. 2) [55]. Similar observations with breast cancer and SKOV3 ovarian cancer models have also been reported [56, 57]. In all the above mentioned studies, the results illustrated tropism of stem cells towards tumors.

In contrast to what we discussed above, there are studies that have argued otherwise and reported that no evidence of MSC migration towards tumors has been observed. For example, in a very interesting study by Bexell et al. (2011), rat bone marrow-derived green fluorescent protein expressing MSCs were injected extra-tumorally in syngeneic rat models of glioma [58]. The authors found no evidence of long-distance MSC migration across the corpus callosum or through the striatum toward malignant gliomas. However, their results suggested that intratumoral implantation may be the method of choice for MSC-based treatment approaches of malignant brain tumors [58].

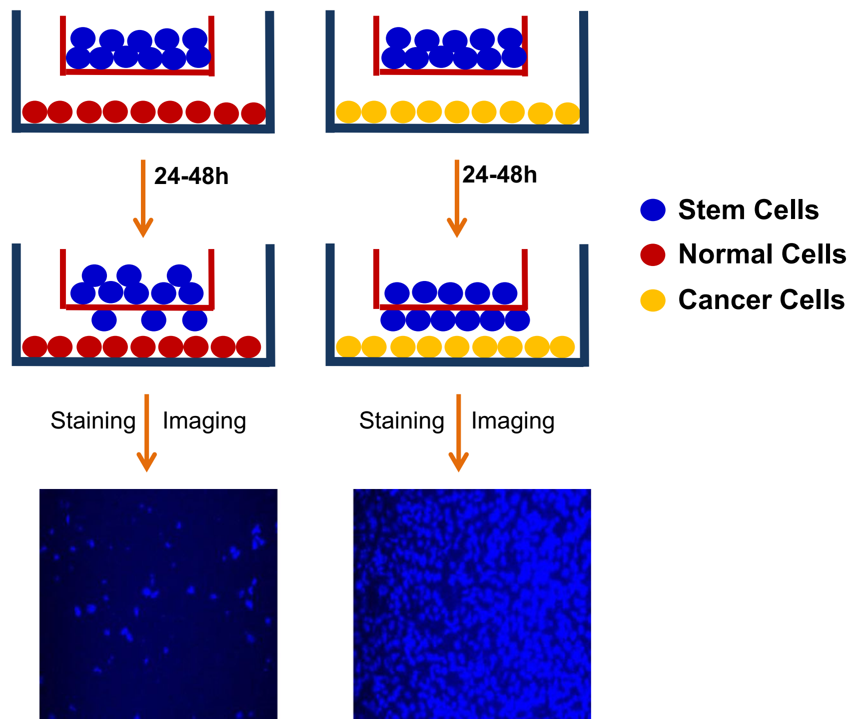
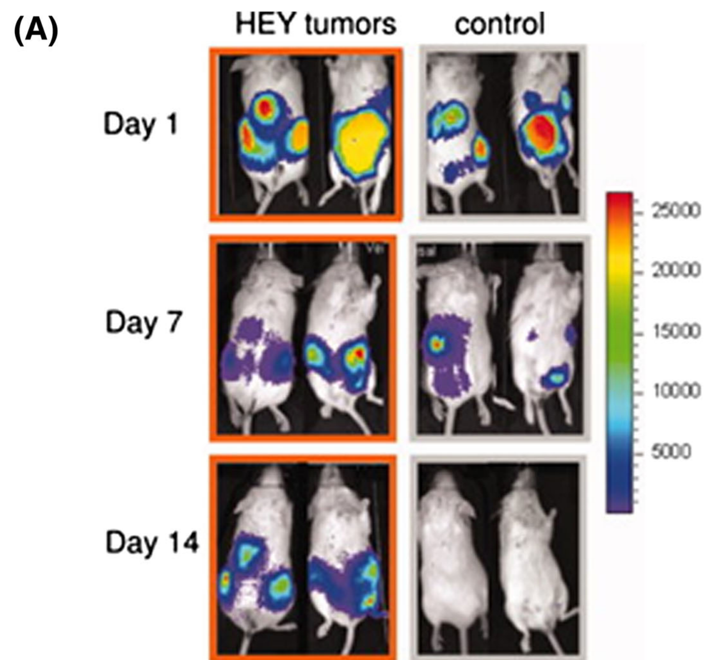


Fig. 1 In Migration assay, stem cells are seeded on the upper layer of a permeable membrane (*upper chamber*) and the cancer cells are seeded in the *bottom chamber*. If tropism exists, it is expected that the stem cells migrate through the membrane towards the tumor cells in the *lower chamber*. A non-cancer cell line is used as control



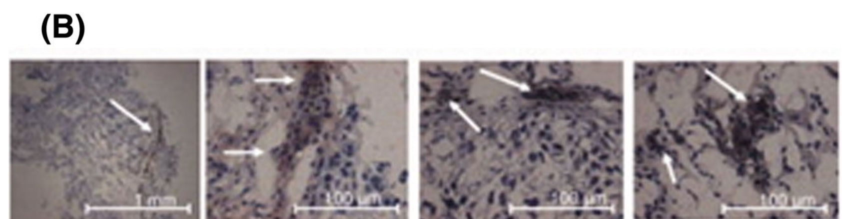


Fig. 2 Tropism of MSCs toward HEY ovarian carcinoma. **a** On day 1, 1×10^6 MSCs were injected into the peritoneal cavity and their localization in tumors was monitored over 14 days. The MSCs which were injected into mice without tumors did not localize; hence, undetectable. **b** Immunohistochemistry study demonstrated the presence of luciferase protein in tumor tissue sections. Adapted with permission from reference [55]

Evaluation of Tumor Tropism of Stem Cells After Intravenous Injection

While injection of the MSCs close to the tumor site has produced promising results, but this approach may not be applicable in many types of cancer due to tumor inaccessibility. The most reliable injection route with widespread application in the clinic is intravenous (IV). After IV injection, it has been shown that stem cells first accumulate in lungs. Understandably, the application of stem cells in treating tumors that are localized in lung has been extensively studied. In a study by Song et al. (2011), lung metastasis models of PC-3 prostate and RIF-1 fibrosarcoma were examined by injecting cancer cells via tail vein to induce lung tumors [3]. Seven days later, rat luciferase expressing bone marrow-derived MSCs (Luc-BMSCs) were injected IV. In both PC-3 and RIF-1 lung metastasis models, Luc-BMSCs were detected mainly in the lung 1 day after injection and remained detectable over a 30 day period. A new batch of BMSCs was then genetically modified to express thymidine kinase enzyme (TK-BMSC) and used in combination with GCV to treat lung tumors. The results demonstrated significant tumor size reduction in lung tumors when TK-BMSCs were administered with GCV. Other groups have also used IV route to inject stem cells and target tumors that are located in the lungs [32, 59]. One important point to highlight is that after IV injection, accumulation of stem cells in lungs is due to their large sizes (~15 μm) and not necessarily their inherent tumor tropism. This is due to the fact that lung is part of reticuloendothelial system and responsible for the entrapment and then clearance of large particles with sizes bigger than 6 μm [60]. Given that tumor tropism of stem cells is dependent on both stem cell lineage and tumor type, it may be somewhat premature at this point to conclude that MSCs have tropism toward all lung tumors [44]. However, there are still considerable numbers of studies which have induced tumors in regions other than lung and have shown tumor tropism of the stem cells after IV injection. For example, in a study by Xia et al. (2011), the tumor tropism was evaluated after IV injection of SPIO-labeled MSCs in mice bearing orthotopic breast xenografts [61]. Using Prussian blue staining technique, the results revealed accumulation of MSCs mainly in tumors and to a significantly less degrees in other organs. This tissue staining technique is also used to illustrate tumor tropism of stem cells in other types of cancers such as human colon cancer [62], primary and metastatic breast cancer [56, 63] and neuroblastoma metastatic tumors [64]. Although tissue sectioning is one way of observing stem cells in various tissues at specific time points, but it does not allow continuous tracking over a long period of time. To overcome this limitation, several groups have used live animal imaging systems to track stem cells inside animals at different time intervals. In a study by Hung et al. (2005), human MSCs were transduced with Herpes Simplex Virus-Thymidine Kinase gene (hMSC-HSVTK) for tracking by Positron Emission Tomography (PET) imaging [65]. HT-29 Inv2 colon carcinoma cells were injected subcutaneous (SC) in the flank region of the SCID mice to induce tumors. After 3 days, 5×10^5 hMSC-HSVTK cells were injected via tail vain. Then Micro PET imaging was performed after infusion of [^{18}F]-FHBG to evaluate the biodistribution of hMSC-HSVTK. Live animal imaging data demonstrated the localization of hMSC-HSVTK cells in the tumors. Later, Yang et al. (2012), studied tumor tropism of IV injected DiR-labeled neural stem cells (NSCs) in immunodeficient NSG and immunocompetent BALB/c mice [66]. Tumors were induced by injecting luciferase expressing 4T1 breast cancer cells in the mammary fat pads. Seven days post tumor induction, DiR-labeled NSCs were injected via tail vein and live animal imaging was performed on days 0, 1, 2, 7, 14. The results displayed the localization of NSCs in 4T1 breast tumor regions within 2 weeks.

In contrast to the studies that we discussed in this section, there are studies with contradictory results which challenge the tumor tropism of the stem cells after IV injection. In a study by Luetzkendorf et al. (2010), TRAIL expressing MSCs were engineered to induce apoptosis in tumor cells [67]. Even though the in vitro co-culture studies and in vivo co-injection of MSCs and tumor cells clearly demonstrated inhibitory effects of stem cells on tumor growth but there was no significant effect after systemic injection of MSCs. Ex vivo studies revealed entrapment of the MSCs in lung and presence of just 0.1 % of stem cells in tumors which was not enough to inhibit tumor growth. Our biodistribution studies with luciferase expressing MSCs in mice model also show their significant accumulation in lungs followed by rapid clearance without any detectable signal in tumors (Fig. 3). This figure indicates that either no MSC could reach the tumors or the number of MSCs

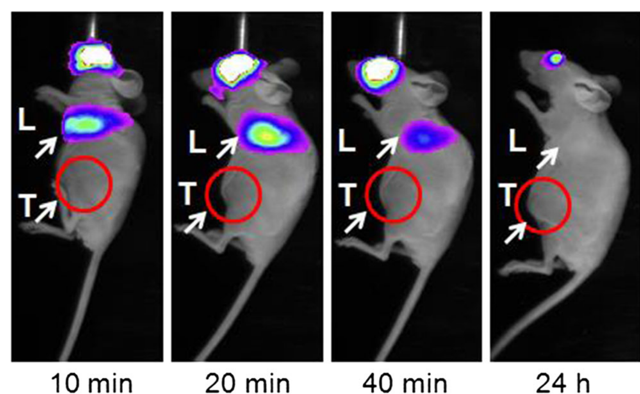


Fig. 3 Use of IVIS animal imaging system to study biodistribution of one million luciferase expressing bone marrow derived MSCs after retroorbital injection in nude mouse. The injected MSCs are first accumulated in the lungs (L) and then disappear after 24 h without any detectable accumulation in tumor (T). The *red circle* identifies the position of tumor

that could reach the tumors was so low that fell below the detection limit of the animal imaging system.

In another study, Eggenhofer et al. (2012), investigated the fate of MSCs after IV infusion [68]. Mouse MSCs expressing DsRed-fluorescent protein and also radioactively labeled with Cr-51 were IV injected in C57BL/6 mice. After 5 min, 1, 24, or 72 h, mice were euthanized and blood, lungs, liver, spleen, kidneys, and bone marrow removed to detect viable MSCs. In vivo and ex vivo tracking studies demonstrated the presence of viable MSCs only in lungs indicating that viable MSCs do not pass lung after IV injection. These results are in agreement with the results of another study which used radiolabeled stem cells to track their biodistribution [69]. In addition to these studies, others have also reported the same outcomes [2]. Such conflicting results in different studies may be indicative of the impact of different factors such as tumor size, tumor type, stem cell source and stem cell passage number on tumor tropism of stem cells [44]. To help identify the determining factors in stem cell tropism, it may be necessary to ask investigators to include all these relevant information in their publications in order to help identify the critical factors that impact stem cells' tumor tropism. It is noteworthy that the degree of MSC entrapment in lungs after i.v. injection may not be as prominent in humans as in mice due to significant physiological and microanatomical differences. Nonetheless, some degree of MSC clearance by lungs is expected even in humans; thereby, this issue must be carefully investigated as it could significantly impact the therapeutic outcome. One approach which could help overcome this hurdle is intra-arterial injection which simply bypasses the lungs. In a study by Doucette et al. (2011), it has been demonstrated that syngeneic bone marrow derived MSCs after intra-arterial injection could effectively localize in tumors and kill the cancer cells while they failed to reach tumors after i.v. administration [70].

Overall, it appears that for effective cancer therapy, significant numbers of stem cells are needed to reach tumors so that they can make an impact on tumor growth. In recent years, several groups have looked at various factors such as radiation, ultra sound, and cell surface modification and their impact on increasing tumor tropism. They have illustrated local radiation or ultrasound exposure of one region can enhance the tropism of the stem cells by increasing the chemokine gradient [71, 72]. Other factors such as cell surface receptors may also play a significant role in stem cell tropism towards tumors. In a study by Nystedt et al. (2013), the cell surface profiles of the MSCs from two origins of bone marrow and umbilical cord blood were compared [73]. They linked the higher lung clearance rate of the umbilical cord blood MSCs to higher expression of the CD49D and CD49f on the cell surfaces. They suggested that modification of the cell surface can be a practical approach to change the lung clearance of the stem cells which is a significant limiting factor to the efficacy of this approach.

So far (as of January 2015), the most successful studies that have reached clinical trials been performed by Aboody's group at the City of Hope where they have used suicide gene expressing neural stem cells for the treatment of glioma (Clinicaltrials.gov, NCT02015819 and NCT01172964). These clinical trials are ongoing and no data have been reported yet.

Tracking the Stem Cells

As the number of stem cells that reach tumors plays a significant role in anti-cancer activity, it is important to validate the stem cell delivery process and quantify the number of stem cells that reach the target so that a reliable dose–response study can be performed. One method that could facilitate such studies is the live imaging of stem cells in vivo. The most broadly used methods for stem cell tracking are bioluminescence imaging (BLI), florescence imaging (FLI), magnetic resonance imaging (MRI) and radionuclide imaging. FLI is performed by exposing a florescent compound to an external light for excitation and is categorized as a high sensitive and non-invasive imaging method. This technique is confined to small animals due to scattering and absorbance of the light by tissues. Ruan et al. (2012), developed murine DiR-labeled embryonic stem cells (DiR-mES) and detected a strong florescence signal within 24 h in vitro [74]. Then, DiR-mES were injected IV to tumor bearing mice and the florescence signal was tracked over a 24 h period. The results revealed the accumulation of the stem cells in tumors. As presented in this study, one of the major problems with using FLI is the short stability of the fluorescent signal due to dilution of the labeling agent with each cell division and particle shedding. Other groups that used quantum dots (QD) for FLI observed similar results in their experiments where the number of QD labeled cells decreased from 72.2 to 4.3 % in a 4 day period [75]. In another study, the percentage of the QD labeled stem cells dropped from 93 to 25 % 3 days post labeling [76]. Overall, it appears that using fluorescent dyes for FLI can be an acceptable approach for tracking stem cells in small animals only when the duration of study is short (<24 h). However, the major disadvantage of FLI with fluorescent dyes is that detection of fluorescent signal under microscope or in animals does not necessarily mean that the cells are alive. As a result, the probability of making wrong conclusions with FLI with fluorescent dyes is high. One approach that could help overcome this shortcoming associated with cell viability is the use of fluorescence-based imaging with MSCs that express fluorescent proteins such as enhanced green or red fluorescent protein family (e.g., EGFP and DsRed) [77]. While use of EGFP and DsRed may be useful in in vitro studies, they may not be as attractive in fluorescence imaging of deep tumors due to limited penetration of light, tissue absorption and

scattering. Recently, Jiguet-Jiglaire et al. (2014), reported the use of an infrared fluorescent protein with fluorescence characteristics laying within a near IR transparency of mammalian tissues [78]. This approach helped overcome issues related to fluorescent light tissue penetration, absorption and scattering in small animals such as mice facilitating more reliable pre-clinical studies; however, the application of this approach in larger animals or humans has not been investigated yet.

One method that has a significant advantage over FLI is BLI. BLI measures the emitted light generated from conversion of a substrate (e.g., luciferin) by an enzyme (luciferase) in live stem cells. This imaging technique has high sensitivity, exclusive to live stem cells and suitable for quantitative studies [79, 80]. For example, our studies demonstrate the ability to image small number of stem cells in mice which could facilitate dose–response studies (Fig. 4) [29]. In addition to our work, others have also used BLI technique to track stem cells in live animals [70, 81]. Wang et al. (2009), modified MSCs to express firefly luciferase in fusion with green fluorescent protein (fLuc-eGFP) to investigate trafficking of the stem cells in 4T1 breast tumor bearing mice [82]. They injected MSCs via tail vein and demonstrated localization of the stem cells in both subcutaneous tumor and lung metastasis model by two dimensional BLI (2D BLI) and histological analysis. The drawback of using 2D BLI is its inability to pinpoint the exact location of bioluminescence source; hence, a complementary histological analysis is needed to identify the anatomical location. In recent years, more refined optical imaging techniques such as three-dimensional BLI (3D BLI) have been utilized which allow us identify the exact source and brightness of bioluminescence foci [83, 84]. While BLI possesses several advantages over FLI, but there are some drawbacks that needs to be considered. One of the shortcomings of this method which has restricted its use to small animals is the absorption and scattering of the emitted light by the tissues and potential immunogenicity of the substrate and enzyme [85]. Therefore, BLI may be a great and reliable technique for performing dose–response studies at the pre-clinical level but for clinical studies other imaging techniques such as MRI are more applicable.

MRI is one of the most commonly used methods in clinic with markers such as gadolinium (Gd3+) and manganese for T1 system and super paramagnetic iron oxide (SPIO) and micron sized particles of iron oxide (MPIOs) for T2 system [86, 87]. The benefits of MRI are high resolution, three dimensional imaging and clinical application. In this imaging technique, stem cells are labeled directly with a contrast agent (e.g., SPIO) or transduced with a gene such as ferritin which can produce magnetic contrast in the cell [88]. The advantage of using ferritin expressing stem cells over labeled ones is that the potential for generation of fake signals by dead cells or engulfed stem cells by scavenger macrophages is eliminated [89]. There are also some studies which have reported the toxic effects of labeling compounds on stem cells properties. Nohroudi et al. (2010), demonstrated that the viability and migratory potential of the BM-MSC decrease as MPIOs incorporation in stem cells increases [90]. In another study, it was shown that SPIO loading of the fetal stem cells impairs cell movements in a dose dependent manner [91]. In terms of application, MRI is broadly used in Glioma cancer model [92–94], although other types of cancer have also been studied. For example Lee et al. (2013), used MRI to track migration of the genetically modified NSCs toward prostate tumors [95]. Despite its significant clinical applications, the drawbacks of using MRI include low sensitivity as compared to the other imaging techniques such as BLI and PET [96], unsuitability for quantitative studies [97] and contraindication in patients with implantable devices.

Currently, methods such as radionuclide imaging provide significant benefits over MRI which makes it suitable for

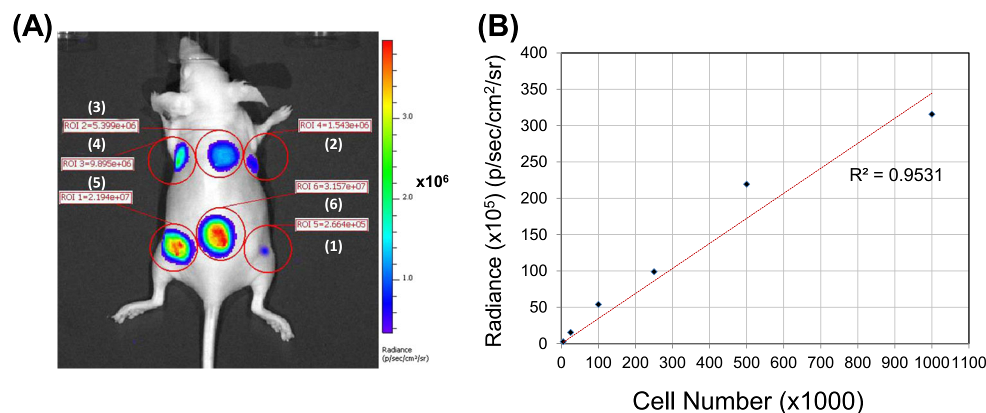


Fig. 4 Bioluminescence imaging. **a** Various number of luciferase expressing stem cells were injected subcutaneously into a nude mouse and then imaged by IVIS live animal imaging system. Numbers 1 to 6 correspond to 5000, 25,000, 100,000, 250,000, 500,000, and 1,000,000 cells, respectively. **b** The luminescence intensity was plotted against cells numbers and a good linear correlation between cell number and luminescence intensity was obtained. Adapted with permission from reference [29]

in vivo tracking of stem cells. These advantages include high sensitivity, application at the clinical level and suitability for quantitative studies [98–100]. Radionuclide imaging employs gamma ray emitting radioisotopes for imaging cells in vivo and contains two types of single photon emission computed tomography (SPECT) and positron emission tomography (PET). While in SPECT radioisotopes send one gamma photon in one direction; in PET radiotracers emit two gamma photons in opposite directions. The most commonly used radiotracers for SPECT are indium-111 (^{111}In) and Technetium-99m (^{99m}Tc), whereas in PET it is usually fluorine-18 (^{18}F) and copper-64 (^{64}Cu). For direct stem cell labeling, ^{18}F can be incorporated into a glucose analog, 2-deoxy-2-^{18}F-fluoro-D-glucose (^{18}F-FDG), or into a modified thymidine analog, 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) both of which could be trapped inside the cells after phosphorylation [101, 102]. These radioisotopes have a short half-life making them suitable for short-term tracking. To extend the stem cells tracking period up to 2 months, they can be genetically modified to express HSV-TK[98]. HSV-TK can phosphorylate and retain pyrimidine analog derivatives such as 2′-fluoro-2′-deoxy-β-D-arabinofuranosyl-5-iodouracil (FIAU) and acycloguanosine derivatives such as 9-(4-fluoro-3-hydroxymethyl-butyl) guanine (FHBG) inside the cells [103, 104]. Another commonly used reporter gene is the sodium iodide symporter (NIS), a trans-membrane protein normally expressed in thyroid cells and responsible for iodine uptake. Stem cells modified to express this transporter are suitable for PET imaging with ^{124}I and SPECT imaging with ^{123}I or ^{99m}Tc. Dwyer et al. (2011), evaluated the use of genetically modified MSCs that could express NIS for imaging and therapy of MDA-MB-231 breast cancer tumors in nude mice [105]. Modified MSCs were injected IV when tumor reached appropriate size and SPECT was performed through use of ^{99m}Tc injection on days 3 and 14. To quantify, accumulation of the radioisotope in each region was calculated and reported as the percentage of the total dose administered. The results revealed enhancement in radiotracer accumulation at the tumor site starting from 1.2 % on day 3 up to 9.4 % on day 14. The results also showed the suitability of the method for quantitative evaluation of the therapeutic efficacy. However, it is important to mention that radiations from radionuclides could induce toxic effects in stem cells and normal tissues. Other drawbacks with the use of radionuclides include release of radiotracers into non-target cells, short half-life of the tracers necessitating repeated injections and lower spatial resolution as compared to MRI [106, 107].

Knowing that each of these imaging techniques has its own advantages and disadvantages, scientists occasionally employ a combination of these methods for tracking stem cells. For example, Wang et al. (2012), used a dual probe approach (Gd^{+} and Cy5.5) for MRI and FLI [108]. Here, MRI provided information regarding spatial distribution of stem cells in tumors, whereas FLI showed presence of stem cells in other non-target organs such as liver with higher sensitivity, a task which could not be achieved by MRI alone because of the homogenous distribution of MSCs.

Conclusion

Overall, it appears that there are two major barriers to effective translation of stem cell-mediated cancer therapy into the clinic. The first barrier is the pulmonary first pass effect which limits the number of viable stem cells that can reach tumors. Since this shortcoming directly impacts the efficacy of the treatment protocols, future studies may need to focus on overcoming this obstacle in order to facilitate translation of this science into the clinic. Perhaps, more emphasis may need to be placed on intra-arterial administration of MSCs rather than intravenous. The second major barrier is related to our limited ability to effectively track the stem cells in vivo. Therefore, it has become increasingly difficult to validate targets and perform reliable dose–response studies. This shortcoming limits our ability to effectively evaluate the therapeutic efficacy and safety of this approach. As genetically modified stem cells can maintain their proliferation capability in vivo, identifying their exact fate after in vivo administration is of paramount importance. It is apparent that there is a lot more work that needs to be done, and significant resources and investments may be required to help overcome these two obstacles in order to make this approach a viable technology for cancer therapy.

Acknowledgments This work was supported in part by grants from the National Institute of Biomedical Imaging and Bioengineering (R21EB016792) and National Cancer Institute (R01CA175318) to A. Hatefi.

Conflict of Interest The authors declare no potential conflicts of interest

References

1. Altaner, C., Altanerova, V., Cihova, M., et al. (2014). Complete regression of glioblastoma by mesenchymal stem cells mediated prodrug gene therapy simulating clinical therapeutic scenario. *International Journal of Cancer, 134*, 1458–1465.
2. Matuskova, M., Hlubinova, K., Pastorakova, A., et al. (2010). HSV-tk expressing mesenchymal stem cells exert bystander effect on human glioblastoma cells. *Cancer Letters, 290*, 58–67.
3. Song, C., Xiang, J., Tang, J., et al. (2011). Thymidine kinase gene modified bone marrow mesenchymal stem cells as vehicles for antitumor therapy. *Human Gene Therapy, 22*, 439–449.
4. Kucerova, L., Matuskova, M., Pastorakova, A., et al. (2008). Cytosine deaminase expressing human mesenchymal stem cells mediated tumour regression in melanoma bearing mice. *The Journal of Gene Medicine, 10*, 1071–1082.

5. Amara, I., Touati, W., Beaune, P., & de Waziers, I. (2014). Mesenchymal stem cells as cellular vehicles for prodrug gene therapy against tumors. *Biochimie, 105*, 4–11.
6. Allen, T. M., & Cullis, P. R. (2004). Drug delivery systems: entering the mainstream. *Science, 303*, 1818–1822.
7. Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R., & Langer, R. (2007). Nanocarriers as an emerging platform for cancer therapy. *Nature Nanotechnology, 2*, 751–760.
8. Yao, X., Yoshioka, Y., Morishige, T., et al. (2009). Systemic administration of a PEGylated adenovirus vector with a cancer-specific promoter is effective in a mouse model of metastasis. *Gene Therapy, 16*, 1395–1404.
9. Yao, X. L., Yoshioka, Y., Ruan, G. X., et al. (2012). Optimization and internalization mechanisms of PEGylated adenovirus vector with targeting peptide for cancer gene therapy. *Biomacromolecules, 13*, 2402–2409.
10. Reddy, L. H. (2005). Drug delivery to tumours: recent strategies. *Journal of Pharmacy and Pharmacology, 57*, 1231–1242.
11. Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*, 6387–6392.
12. Lammers, T., Kiessling, F., Hennink, W. E., & Storm, G. (2012). Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. *Journal of Controlled Release, 161*, 175–187.
13. Zhao, D., Najbauer, J., Garcia, E., et al. (2008). Neural stem cell tropism to glioma: critical role of tumor hypoxia. *Molecular Cancer Research, 6*, 1819–1829.
14. Teo, G. S., Ankrum, J. A., Martinelli, R., et al. (2012). Mesenchymal stem cells transmigrate between and directly through tumor necrosis factor-alpha-activated endothelial cells via both leukocyte-like and novel mechanisms. *Stem Cells, 30*, 2472–2486.
15. Studeny, M., Marini, F. C., Dembinski, J. L., et al. (2004). Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *Journal of the National Cancer Institute, 96*, 1593–1603.
16. Ren, C., Kumar, S., Chanda, D., et al. (2008). Cancer gene therapy using mesenchymal stem cells expressing interferon-beta in a mouse prostate cancer lung metastasis model. *Gene Therapy, 15*, 1446–1453.
17. Ahn, J., Lee, H., Seo, K., Kang, S., Ra, J., & Youn, H. (2013). Anti-tumor effect of adipose tissue derived-mesenchymal stem cells expressing interferon-beta and treatment with cisplatin in a xenograft mouse model for canine melanoma. *PLoS ONE, 8*, e74897.
18. Nakamizo, A., Marini, F., Amano, T., et al. (2005). Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Research, 65*, 3307–3318.
19. Nakamura, K., Ito, Y., Kawano, Y., et al. (2004). Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. *Gene Therapy, 11*, 1155–1164.
20. Stagg, J., Lejeune, L., Paquin, A., & Galipeau, J. (2004). Marrow stromal cells for interleukin-2 delivery in cancer immunotherapy. *Human Gene Therapy, 15*, 597–608.
21. Moniri, M. R., Sun, X. Y., Rayat, J., et al. (2012). TRAIL-engineered pancreas-derived mesenchymal stem cells: characterization and cytotoxic effects on pancreatic cancer cells. *Cancer Gene Therapy, 19*, 652–658.
22. Ciavarella, S., Grisendi, G., Dominici, M., et al. (2012). In vitro anti-myeloma activity of TRAIL-expressing adipose-derived mesenchymal stem cells. *British Journal of Haematology, 157*, 586–598.
23. Khan, Z., Knecht, W., Willer, M., et al. (2010). Plant thymidine kinase 1: a novel efficient suicide gene for malignant glioma therapy. *Neuro-Oncology, 12*, 549–558.
24. Gu, C., Li, S., Tokuyama, T., Yokota, N., & Namba, H. (2010). Therapeutic effect of genetically engineered mesenchymal stem cells in rat experimental leptomeningeal glioma model. *Cancer Letters, 291*, 256–262.
25. Kosaka, H., Ichikawa, T., Kurozumi, K., et al. (2012). Therapeutic effect of suicide gene-transferred mesenchymal stem cells in a rat model of glioma. *Cancer Gene Therapy, 19*, 572–578.
26. Kucerova, L., Matuskova, M., Hlubinova, K., et al. (2011). Bystander cytotoxicity in human medullary thyroid carcinoma cells mediated by fusion yeast cytosine deaminase and 5-fluorocytosine. *Cancer Letters, 311*, 101–112.
27. Studeny, M., Marini, F. C., Champlin, R. E., Zompetta, C., Fidler, I. J., & Andreeff, M. (2002). Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. *Cancer Research, 62*, 3603–3608.
28. Uhl, M., Weiler, M., Wick, W., Jacobs, A. H., Weller, M., & Herrlinger, U. (2005). Migratory neural stem cells for improved thymidine kinase-based gene therapy of malignant gliomas. *Biochemical and Biophysical Research Communications, 328*, 125–129.
29. Nouri, F. S., Wang, X., & Hatefi, A. (2015). Genetically engineered theranostic mesenchymal stem cells for the evaluation of the anticancer efficacy of enzyme/prodrug systems. *Journal of Controlled Release, 200*, 179–187.
30. Benedetti, S., Pirola, B., Pollo, B., et al. (2000). Gene therapy of experimental brain tumors using neural progenitor cells. *Nature Medicine, 6*, 447–450.
31. Kyriakou, C. A., Yong, K. L., Benjamin, R., et al. (2006). Human mesenchymal stem cells (hMSCs) expressing truncated soluble vascular endothelial growth factor receptor (tsFlk-1) following lentiviral-mediated gene transfer inhibit growth of Burkitt's lymphoma in a murine model. *The Journal of Gene Medicine, 8*, 253–264.
32. Loebinger, M. R., Eddaoudi, A., Davies, D., & Janes, S. M. (2009). Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. *Cancer Research, 69*, 4134–4142.
33. van de Water, J. A., Bagci-Onder, T., Agarwal, A. S., et al. (2012). Therapeutic stem cells expressing variants of EGFR-specific nanobodies have antitumor effects. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 16642–16647.
34. Secchiero, P., Zorzet, S., Tripodo, C., et al. (2010). Human bone marrow mesenchymal stem cells display anti-cancer activity in SCID mice bearing disseminated non-Hodgkin's lymphoma xenografts. *PLoS ONE, 5*, e11140.
35. Lu, Y. R., Yuan, Y., Wang, X. J., et al. (2008). The growth inhibitory effect of mesenchymal stem cells on tumor cells in vitro and in vivo. *Cancer Biology & Therapy, 7*, 245–251.
36. Cousin, B., Ravet, E., Poglio, S., et al. (2009). Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. *PLoS ONE, 4*, e6278.
37. Sun, B., Roh, K. H., Park, J. R., et al. (2009). Therapeutic potential of mesenchymal stromal cells in a mouse breast cancer metastasis model. *Cytotherapy, 11*, 289–298.
38. Keramidas, M., de Fraipont, F., Karageorgis, A., et al. (2013). The dual effect of mesenchymal stem cells on tumour growth and tumour angiogenesis. *Stem Cell Research & Therapy, 4*, 41.
39. Shinagawa, K., Kitadai, Y., Tanaka, M., et al. (2010). Mesenchymal stem cells enhance growth and metastasis of colon cancer. *International Journal of Cancer, 127*, 2323–2333.
40. Prantl, L., Muehlberg, F., Navone, N. M., et al. (2010). Adipose tissue-derived stem cells promote prostate tumor growth. *Prostate, 70*, 1709–1715.
41. Galie, M., Konstantinidou, G., Peroni, D., et al. (2008). Mesenchymal stem cells share molecular signature with

mesenchymal tumor cells and favor early tumor growth in syngeneic mice. *Oncogene, 27*, 2542–2551.

42. Klopp, A. H., Gupta, A., Spaeth, E., Andreeff, M., & Marini, F., 3rd. (2011). Concise review: Dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? *Stem Cells, 29*, 11–19.
43. Salem, H. K., & Thiemermann, C. (2010). Mesenchymal stromal cells: current understanding and clinical status. *Stem Cells, 28*, 585–596.
44. Belmar-Lopez, C., Mendoza, G., Oberg, D., et al. (2013). Tissue-derived mesenchymal stromal cells used as vehicles for anti-tumor therapy exert different in vivo effects on migration capacity and tumor growth. *BMC Medicine, 11*, 139.
45. Ringe, J., Strassburg, S., Neumann, K., et al. (2007). Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. *Journal of Cellular Biochemistry, 101*, 135–146.
46. Ponte, A. L., Marais, E., Gallay, N., et al. (2007). The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells, 25*, 1737–1745.
47. Pendleton, C., Li, Q., Chesler, D. A., Yuan, K., Guerrero-Cazares, H., & Quinones-Hinojosa, A. (2013). Mesenchymal stem cells derived from adipose tissue vs bone marrow: in vitro comparison of their tropism towards gliomas. *PLoS ONE, 8*, e58198.
48. Frank, R. T., Edmiston, M., Kendall, S. E., et al. (2009). Neural stem cells as a novel platform for tumor-specific delivery of therapeutic antibodies. *PLoS ONE, 4*, e8314.
49. Sasportas, L. S., Kasmieh, R., Wakimoto, H., et al. (2009). Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 4822–4827.
50. Mercapide, J., Rappa, G., Anzanello, F., King, J., Fodstad, O., & Lorico, A. (2010). Primary gene-engineered neural stem/progenitor cells demonstrate tumor-selective migration and antitumor effects in glioma. *International Journal of Cancer, 126*, 1206–1215.
51. Kim, J. H., Kim, J. Y., Kim, S. U., & Cho, K. G. (2012). Therapeutic effect of genetically modified human neural stem cells encoding cytosine deaminase on experimental glioma. *Biochemical and Biophysical Research Communications, 417*, 534–540.
52. Li, S., Gao, Y., Tokuyama, T., et al. (2007). Genetically engineered neural stem cells migrate and suppress glioma cell growth at distant intracranial sites. *Cancer Letters, 251*, 220–227.
53. Zhao, Y., Lam, D. H., Yang, J., et al. (2012). Targeted suicide gene therapy for glioma using human embryonic stem cell-derived neural stem cells genetically modified by baculoviral vectors. *Gene Therapy, 19*, 189–200.
54. Lee, D. H., Ahn, Y., Kim, S. U., et al. (2009). Targeting rat brainstem glioma using human neural stem cells and human mesenchymal stem cells. *Clinical Cancer Research, 15*, 4925–4934.
55. Kidd, S., Spaeth, E., Dembinski, J. L., et al. (2009). Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging. *Stem Cells, 27*, 2614–2623.
56. Dwyer, R. M., Potter-Beirne, S. M., Harrington, K. A., et al. (2007). Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. *Clinical Cancer Research, 13*, 5020–5027.
57. Komarova, S., Kawakami, Y., Stoff-Khalili, M. A., Curiel, D. T., & Pereboeva, L. (2006). Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. *Molecular Cancer Therapeutics, 5*, 755–766.
58. Bexell, D., Gunnarsson, S., Svensson, A., et al. (2012). Rat multipotent mesenchymal stromal cells lack long-distance tropism to 3 different rat glioma models. *Neurosurgery, 70*, 731–739.
59. Kim, S. W., Kim, S. J., Park, S. H., et al. (2013). Complete regression of metastatic renal cell carcinoma by multiple injections of engineered mesenchymal stem cells expressing dodecameric TRAIL and HSV-TK. *Clinical Cancer Research, 19*, 415–427.
60. Chao, P., Deshmukh, M., Kutscher, H. L., et al. (2010). Pulmonary targeting microparticulate camptothecin delivery system: anticancer evaluation in a rat orthotopic lung cancer model. *Anti-Cancer Drugs, 21*, 65–76.
61. Xia, X., Ji, T., Chen, P., et al. (2011). Mesenchymal stem cells as carriers and amplifiers in CRAd delivery to tumors. *Molecular Cancer, 10*, 134.
62. Kucerova, L., Altanerova, V., Matuskova, M., Tyciakova, S., & Altaner, C. (2007). Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. *Cancer Research, 67*, 6304–6313.
63. Zhao, D., Najbauer, J., Annala, A. J., et al. (2012). Human neural stem cell tropism to metastatic breast cancer. *Stem Cells, 30*, 314–325.
64. Aboody, K. S., Bush, R. A., Garcia, E., et al. (2006). Development of a tumor-selective approach to treat metastatic cancer. *PLoS ONE, 1*, e23.
65. Hung, S. C., Deng, W. P., Yang, W. K., et al. (2005). Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. *Clinical Cancer Research, 11*, 7749–7756.
66. Yang, J., Lam, D. H., Goh, S. S., et al. (2012). Tumor tropism of intravenously injected human-induced pluripotent stem cell-derived neural stem cells and their gene therapy application in a metastatic breast cancer model. *Stem Cells, 30*, 1021–1029.
67. Luetzkendorf, J., Mueller, L. P., Mueller, T., Caysa, H., Nerger, K., & Schmoll, H. J. (2010). Growth inhibition of colorectal carcinoma by lentiviral TRAIL-transgenic human mesenchymal stem cells requires their substantial intratumoral presence. *Journal of Cellular and Molecular Medicine, 14*, 2292–2304.
68. Eggenhofer, E., Benseler, V., Kroemer, A., et al. (2012). Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Frontiers in Immunology, 3*, 297.
69. Mukherjee, A., Tipnis, S., Sarma, H. D., et al. (2012). Radiolabeling of umbilical cord-derived mesenchymal stem cells for in vivo tracking. *Cancer Biotherapy and Radiopharmaceuticals, 27*, 614–619.
70. Doucette, T., Rao, G., Yang, Y., et al. (2011). Mesenchymal stem cells display tumor-specific tropism in an RCAS/Ntv-a glioma model. *Neoplasia, 13*, 716–725.
71. Kim, S. M., Oh, J. H., Park, S. A., et al. (2010). Irradiation enhances the tumor tropism and therapeutic potential of tumor necrosis factor-related apoptosis-inducing ligand-secreting human umbilical cord blood-derived mesenchymal stem cells in glioma therapy. *Stem Cells, 28*, 2217–2228.
72. Ziadloo, A., Burks, S. R., Gold, E. M., et al. (2012). Enhanced homing permeability and retention of bone marrow stromal cells by noninvasive pulsed focused ultrasound. *Stem Cells, 30*, 1216–1227.
73. Nystedt, J., Anderson, H., Tikkanen, J., et al. (2013). Cell surface structures influence lung clearance rate of systemically infused mesenchymal stromal cells. *Stem Cells, 31*, 317–326.
74. Ruan, J., Song, H., Li, C., et al. (2012). DiR-labeled Embryonic Stem Cells for Targeted Imaging of in vivo Gastric Cancer Cells. *Theranostics, 2*, 618–628.
75. Lin, S., Xie, X., Patel, M. R., et al. (2007). Quantum dot imaging for embryonic stem cells. *BMC Biotechnology, 7*, 67.

76. Muller-Borer, B. J., Collins, M. C., Gunst, P. R., Cascio, W. E., & Kypson, A. P. (2007). Quantum dot labeling of mesenchymal stem cells. *Journal of Nanobiotechnology, 5*, 9.
77. Bouvet, M., Wang, J., Nardin, S. R., et al. (2002). Real-time optical imaging of primary tumor growth and multiple metastatic events in a pancreatic cancer orthotopic model. *Cancer Research, 62*, 1534–1540.
78. Jiguet-Jiglaire, C., Cayol, M., Mathieu, S., et al. (2014). Noninvasive near-infrared fluorescent protein-based imaging of tumor progression and metastases in deep organs and intraosseous tissues. *Journal of Biomedical Optics, 19*, 16019.
79. Togel, F., Yang, Y., Zhang, P., Hu, Z., & Westenfelder, C. (2008). Bioluminescence imaging to monitor the in vivo distribution of administered mesenchymal stem cells in acute kidney injury. *American Journal of Physiology. Renal Physiology, 295*, F315–F321.
80. Close, D. M., Xu, T., Sayler, G. S., & Ripp, S. (2011). In vivo bioluminescent imaging (BLI): noninvasive visualization and interrogation of biological processes in living animals. *Sensors (Basel), 11*, 180–206.
81. Yan, C., Li, S., Li, Z., et al. (2013). Human umbilical cord mesenchymal stem cells as vehicles of CD20-specific TRAIL fusion protein delivery: a double-target therapy against non-Hodgkin's lymphoma. *Molecular Pharmaceutics, 10*, 142–151.
82. Wang, H., Cao, F., De, A., et al. (2009). Trafficking mesenchymal stem cell engraftment and differentiation in tumor-bearing mice by bioluminescence imaging. *Stem Cells, 27*, 1548–1558.
83. Collins, J.W., Meganck, J.A., Kuo, C., Francis, K.P., Frankel, G. (2013). 4D multimodality imaging of Citrobacter rodentium infections in mice. Journal of Visualized Experiments.
84. Kuo, C., Coquoz, O., Troy, T. L., Xu, H., & Rice, B. W. (2007). Three-dimensional reconstruction of in vivo bioluminescent sources based on multispectral imaging. *Journal of Biomedical Optics, 12*, 024007.
85. Li, S. C., Tachiki, L. M., Luo, J., Dethlefs, B. A., Chen, Z., & Loudon, W. G. (2010). A biological global positioning system: considerations for tracking stem cell behaviors in the whole body. *Stem Cell Reviews, 6*, 317–333.
86. Aoki, I., Wu, Y. J. L., Silva, A. C., Lynch, R. M., & Koretsky, A. P. (2004). In vivo detection of neuroarchitecture in the rodent brain using manganese-enhanced MRI. *NeuroImage, 22*, 1046–1059.
87. Zhang, S. J., & Wu, J. C. (2007). Comparison of imaging techniques for tracking cardiac stem cell therapy. *Journal of Nuclear Medicine, 48*, 1916–1919.
88. Liu, J., Cheng, E. C., Long, R. C., et al. (2009). Noninvasive monitoring of embryonic stem cells in vivo with MRI transgene reporter. *Tissue Engineering. Part C, Methods, 15*, 739–747.
89. Li, Z., Suzuki, Y., Huang, M., et al. (2008). Comparison of reporter gene and iron particle labeling for tracking fate of human embryonic stem cells and differentiated endothelial cells in living subjects. *Stem Cells, 26*, 864–873.
90. Nohroudi, K., Arnhold, S., Berhorn, T., Addicks, K., Hoehn, M., & Himmelreich, U. (2010). In vivo MRI stem cell tracking requires balancing of detection limit and cell viability. *Cell Transplantation, 19*, 431–441.
91. Diana, V., Bossolasco, P., Moscatelli, D., Silani, V., & Cova, L. (2013). Dose dependent side effect of superparamagnetic iron oxide nanoparticle labeling on cell motility in two fetal stem cell populations. *PLoS ONE, 8*, e78435.
92. Brekke, C., Williams, S. C., Price, J., Thorsen, F., & Modo, M. (2007). Cellular multiparametric MRI of neural stem cell therapy in a rat glioma model. *NeuroImage, 37*, 769–782.
93. Wu, X., Hu, J., Zhou, L., et al. (2008). In vivo tracking of superparamagnetic iron oxide nanoparticle-labeled mesenchymal stem cell tropism to malignant gliomas using magnetic resonance imaging. Laboratory investigation. *Journal of Neurosurgery, 108*, 320–329.
94. Thu, M. S., Najbauer, J., Kendall, S. E., et al. (2009). Iron labeling and pre-clinical MRI visualization of therapeutic human neural stem cells in a murine glioma model. *PLoS ONE, 4*, e7218.
95. Lee, H. J., Doo, S. W., Kim, D. H., et al. (2013). Cytosine deaminase-expressing human neural stem cells inhibit tumor growth in prostate cancer-bearing mice. *Cancer Letters, 335*, 58–65.
96. Boersma, H. H., Tromp, S. C., Hofstra, L., & Narula, J. (2005). Stem cell tracking: reversing the silence of the lambs. *Journal of Nuclear Medicine, 46*, 200–203.
97. Daldrup-Link, H. E., Rudelius, M., Metz, S., et al. (2004). Cell tracking with gadophrin-2: a bifunctional contrast agent for MR imaging, optical imaging, and fluorescence microscopy. *European Journal of Nuclear Medicine and Molecular Imaging, 31*, 1312–1321.
98. Love, Z., Wang, F., Dennis, J., et al. (2007). Imaging of mesenchymal stem cell transplant by bioluminescence and PET. *Journal of Nuclear Medicine, 48*, 2011–2020.
99. Gera, A., Steinberg, G. K., & Guzman, R. (2010). In vivo neural stem cell imaging: current modalities and future directions. *Regenerative Medicine, 5*, 73–86.
100. Jiang, H., Cheng, Z., Tian, M., & Zhang, H. (2011). In vivo imaging of embryonic stem cell therapy. *European Journal of Nuclear Medicine and Molecular Imaging, 38*, 774–784.
101. Hofmann, M., Wollert, K. C., Meyer, G. P., et al. (2005). Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation, 111*, 2198–2202.
102. Gu, E., Chen, W. Y., Gu, J., Burridge, P., & Wu, J. C. (2012). Molecular imaging of stem cells: tracking survival, biodistribution, tumorigenicity, and immunogenicity. *Theranostics, 2*, 335–345.
103. Miletic, H., Fischer, Y., Litwak, S., et al. (2007). Bystander killing of malignant glioma by bone marrow-derived tumor-infiltrating progenitor cells expressing a suicide gene. *Molecular Therapy, 15*, 1373–1381.
104. Zhang, G., Lan, X., Yen, T. C., et al. (2012). Therapeutic gene expression in transduced mesenchymal stem cells can be monitored using a reporter gene. *Nuclear Medicine and Biology, 39*, 1243–1250.
105. Dwyer, R. M., Ryan, J., Havelin, R. J., et al. (2011). Mesenchymal Stem Cell-mediated delivery of the sodium iodide symporter supports radionuclide imaging and treatment of breast cancer. *Stem Cells, 29*, 1149–1157.
106. Frangioni, J. V., & Hajjar, R. J. (2004). In vivo tracking of stem cells for clinical trials in cardiovascular disease. *Circulation, 110*, 3378–3383.
107. Stojanov, K., de Vries, E. F., Hoekstra, D., van Waarde, A., Dierckx, R. A., & Zuhorn, I. S. (2012). [18F] FDG labeling of neural stem cells for in vivo cell tracking with positron emission tomography: inhibition of tracer release by phloretin. *Molecular Imaging, 11*, 1–12.
108. Wang, X. Y., Ju, S., Li, C., et al. (2012). Non-invasive imaging of endothelial progenitor cells in tumor neovascularization using a novel dual-modality paramagnetic/near-infrared fluorescence probe. *PLoS ONE, 7*, e50575.