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MRI Reporter Genes: Application to Imaging of Cell Survival, Proliferation, Migration, and Differentiation

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Abstract

Molecular imaging strives to detect molecular events at the level of the whole organism. In some cases, the molecule of interest can be detected either directly, or through the use of targeted contrast media. However many genes and proteins, and particularly those located in intracellular compartments, are not accessible for targeted agents. The transcriptional regulation of these genes can never the less be detected, though indirectly, through the use of reporter genes encoding for readily detectable proteins. Such reporter proteins can be expressed in the tissue of interest by genetically introducing the reporter gene in the target cells. Imaging of reporter genes has become a powerful tool in modern biomedical research. Typically, expression of fluorescent or bioluminescent proteins, or the reaction product of expressed enzymes and exogenous substrates, are examined using *in vitro* histological methods, or *in vivo* whole body imaging methods. Recent advances in MRI reporter gene methods raise the possibility that MRI could become a powerful tool for concomitant high resolution anatomical and functional imaging and for imaging of reporter gene activity. An immediate application of MRI reporter gene methods is for monitoring gene expression patterns in gene therapy and for *in vivo* imaging of the survival, proliferation, migration, and differentiation of pluripotent or multipotent cells used in cell based regenerative therapies for cancer, myocardial infarction, and neural degeneration. In this review, we characterize the variety of MRI reporter gene methods based on their applicability to report cell survival/proliferation, cell migration, and cell differentiation. In particular, we discuss which methods are best suited for translation to clinical use in regenerative therapies.

Keywords

MRI Reporter Gene; Proliferation; Migration; Differentiation; Ferritin; β -galactosidase; CEST; Transferrin Receptor; Iron Oxide Nanoparticles

Introduction

Over the last twenty years, reporter genes have become ubiquitous in basic biomedical research. Reporter genes are generated when the regulatory promoter from a gene of interest is fused to the gene of either an easily detectable protein, or of an enzyme capable of generating detectable contrast upon reaction with an exogenously delivered substrate. Generally, a reporter gene is chosen on the basis of the convenience, sensitivity, dynamic range and reliability of its assay, and the inertness of its expression. Visualization of reporter genes is typically accomplished by one of two mechanisms: (a) detection of the reporter's

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Conflicts of Interest

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endogenous signal: for example, expression of a fluorescent protein such as green fluorescent protein (GFP) which can be directly detected through its endogenous fluorescence upon illumination (1), or (b) detection of signal upon activation or retention of an exogenously administered substrate: for example, expression of an enzyme such as firefly luciferase or β -galactosidase (β -gal) and exogenous delivery of the substrate, so as to yield a detectable reaction product. Applications of reporter genes can vary from elucidating the roles of specific genes in disease processes, mapping the fate of specific precursor cells in development, monitoring migration, proliferation, and differentiation of exogenously administered cells in regenerative medicine, and assessing the efficacy of gene therapies.

Traditionally, the expression of reporter genes has been examined *ex vivo* using histological or immunohistochemical staining techniques (for reporter genes such as beta-galactosidase), or *in vivo* using fluorescent (1) and luminescent (2) imaging (for reporter genes such as the green fluorescent protein or the light emitting reaction catalyzed by the firefly luciferase, respectively). While *ex vivo* methods provide high spatial resolution, they lack individual dynamic information and require the sacrifice of large numbers of animals in order to complete longitudinal studies. In contrast, *in vivo* optical imaging methods such as fluorescence or bioluminescence imaging show little anatomical information, and the sensitivity and spatial resolution deteriorate fast in deeper tissues due to light scattering.

The ability to acquire high quality whole body anatomical images at superior spatial resolutions with magnetic resonance imaging (MRI) is well established in both preclinical research and in clinical settings. Over the last decade, a number of MRI reporter genes were investigated in the context of cardiac, cancer, and neurological research (3, 4). Despite large differences in biomedical questions, these fields share the need to image cell survival and proliferation, cell migration, and cell differentiation. A number of MRI reporter genes methods have been developed to address these needs, ranging from constitutive over-expression of iron binding proteins to expression of plasma membrane peptides that can be targeted with appropriately conjugated contrast agents. To date, most of the *in vivo* MRI reporter gene studies have been performed for imaging of cell survival and proliferation (Table 1). The aim of this review will be to highlight the ability to detect, in the living animal, intracellular decisions such as proliferation, migration and differentiation through the use of ^1H -MRI reporter genes in the context of translational cardiovascular, neurological, and cancer research.

I. The Molecular Biology of Reporter Genes

Reporter genes are molecularly engineered tools that enable assessment of location, duration, and extent of transcriptional activity of a specific promoter within living cells or whole organisms. Reporter constructs can be used to probe signaling pathways, post-transcriptional modifications, protein-protein interactions, protein stability, translocation and cell trafficking. Typically, reporter gene constructs include a cassette with a desired marker gene and a promoter of interest with requisite transcriptional control elements that initiate and regulate expression. Reporter gene expression can be constitutive, inducible, or tissue specific depending on the endogenous activity of the promoter. Promoters that are used to generate continuous transcription are best suited for monitoring the level and duration of gene activity, the survival of transgenic cells, and the migration of transgenic cells. In contrast, inducible promoters can sense endogenous changes in gene expression and provide information about processes such as cell differentiation, as well as initiation and progression of disease. The most widely used reporter genes for *in vivo* imaging include fluorescent (1) and bioluminescent proteins (2). Imaging of both fluorescent and bioluminescent reporter proteins is characterized by high sensitivity, and the ability to image numerous reporter proteins in the same imaging session. However, optical imaging techniques are limited by

the high scattering of light in tissues leading to loss of spatial resolution, and sensitivity to the anatomical depth of signal generation. These reporter genes have limited potential for whole body clinical imaging, though they would be the methods of choice for guidance of invasive procedures including surgery and endoscopy.

II. Mechanisms of MRI Contrast

MRI is renowned for generating anatomical images with superior soft-tissue contrast and high spatial resolution. Typically, contrast on MR images is generated by tailoring pulse sequences to exploit differences in either proton density, or tissue relaxation properties. The relaxation processes governing MRI are defined as spin-lattice (T1) relaxation, which characterizes the return of nuclear spins to the equilibrium state along the direction of the static magnetic field (B_0), and spin-spin (T2) relaxation, which characterizes the loss of spin coherence and net magnetization in the transverse plane after radio frequency (RF) excitation. Additionally, the combined effects of B_0 -inhomogeneities, susceptibility artifacts, and chemical shifts (collectively termed off-resonance effects) during imaging result in a hastened decay of coherent transverse magnetization, termed T2* decay. MRI contrast agents typically act to hasten either T1 or T2 relaxation in order to produce hyper or hypo-intensities (respectively) on appropriately weighted images. ‘Positive’ contrast agents such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}) typically shorten T1 relaxation by direct interaction of unpaired electrons in the outer shells of the paramagnetic metal ion with nearby water molecules. In juxtaposition, ‘negative’ contrast agents, such as the family of super-paramagnetic iron oxide (SPIO) nanoparticles (5), typically induce localized field inhomogeneities that accelerate T2 relaxation and T2* decay. Additional mechanisms for generating specific contrast such as chemical exchange and chemical shift have also been exploited for the design and detection of the various reporter genes.

III. MRI Reporter Genes for Imaging of Cell Survival and Proliferation

Imaging of cell survival and proliferation is a common requirement in biomedical research, ranging from pre-clinical cancer models to translational regenerative therapies for cardiac and neurological diseases. Alongside increased application of MRI for anatomical and functional imaging, a number of MRI reporter gene methods have been developed to enable *in vivo* imaging of cell survival and proliferation. Specifically, one approach has been to use constitutive over-expression of iron binding proteins in cells, such as the transferrin receptor (TfR) (6, 7) and ferritin (8–14), similar to constitutive expression of GFP, to generate T2/T2* contrast on MR images. A parallel approach has been to use transgenic cells that express the LacZ gene, which encodes for β -gal, and to use β -gal substrate analogs, which upon activation yield MRI detectable contrast agents (15–20). A more recent approach has been to use MRI detectable targeted contrast media for detection of engineered cell surface reporter genes (21–28). And finally, tailored artificial peptides making use of the chemical shift sensitivity of chemical exchange saturation transfer (CEST)-MRI have recently emerged as novel MRI reporter genes (29–33).

Constitutive expression of fluorescent reporter genes, which has become omnipresent in pre-clinical biological research (1), enables imaging of cell survival and proliferation by producing strong contrast between expressing cells and background cells on fluorescent images. Early research into MRI reporter genes attempted to mimic this behavior by using over-expression of an enzyme catalyzing a reaction that yielded a detectable product capable of producing strong contrast compared to background cells on MR images. For example, the discovery that heightened tyrosinase activity in melanotic melanomas resulted in enhanced signal intensity on T1-weighted MR images in patients prompted some to examine over-expression of tyrosinase as an MRI reporter gene for tracking of cell survival (34). Early

studies demonstrated increased MRI signal intensity in samples of murine fibroblasts and human kidney cells transfected to over-express human tyrosinase (34). This finding was confirmed in two subsequent studies that used conditional expression of tyrosinase in human breast cancer cells (35, 36).

A more widely examined option in the search for MRI reporter genes has involved members of the family of proteins responsible for maintenance of iron homeostasis. Free labile iron is highly reactive, as it catalyzes the Fenton reaction to generate hydroxyl radicals. Thus the level of free iron in cells is tightly controlled by reciprocal activation or suppression of transcription and protein stability of iron uptake via the transferrin receptor (TfR), and iron storage in ferritin through specific iron responsive elements (IRE's). Amongst the potential candidates, the TfR was the first reporter gene to be evaluated (6, 25). When over-expressed by cells, TfR lead to a small but significant enhancement of R2* contrast (6). However, TfR associated contrast was markedly enhanced through the use of transferrin labeled contrast media (25). An alternative approach was demonstrated for *in vivo* adenoviral mediated infection of neurons to over-express both the heavy and light chains of ferritin (FHC and FLC, respectively) (9, 37). In addition, the capacity of MRI using ferritin as a reporter gene to detect promoter specific activation of transcription was demonstrated in early studies of transgenic mice with conditional and inducible over expression of FHC for specific cell populations such as endothelial cells in the brain or fetal organs (37, 38), or in liver hepatocytes (37, 38). In total, studies from multiple laboratories have used over-expression of either the FHC (8, 10, 11, 14, 37, 39), both the FHC and FLC (9), or the TfR (6, 7), in order to increase endogenous intra-cellular iron storage within over-expressing cells (see illustration in Figure 1A). Importantly, when trying to alter the iron homeostasis in cells using reporter genes which are members of the native iron uptake and storage pathway (TfR, FHC, LHC), it is critical to make sure that the endogenous IRE's are not included in the construct.

Amongst studies which used ferritin over-expression to monitor cell survival and proliferation in pre-clinical rodent models, one study used cardiospheres composed of FHC over-expressing swine cardiac stem/progenitor cells that were injected into infarcted myocardium following myocardial infarction (MI) in rats (14). The long-term survival of the cardiospheres was assessed using T2* weighted imaging and T2* mapping (14). Several studies in rodent cancer models have used over-expression of FHC to image the proliferation of murine melanoma cells after implantation near major lymph nodes (13), or proliferation of human breast cancer and rat glioma cells after sub-dermal implantation in mice and rats (11). While such studies in pre-clinical cardiovascular and cancer models utilized concentrated injections of large numbers of FHC over-expressing cells (millions), typical injections in neurological studies utilize orders of magnitude less cells (thousands), presenting a potential sensitivity barrier to the use of ferritin as an MRI reporter gene for neurological studies. In order to address this limitation, Deans et al. used coupled over-expression of both FHC and TfR in murine neural stem cells (7). Acquisition of T2*-weighted images ten days after bolus injection of 2500 cells into the mouse brain demonstrated clear hypo-intensity around the site of injection (7)(Figure 1B).

Exposing wild type or ferritin over expressing cells or mice to elevated iron in the culture medium or food, as was done in the aforementioned studies, enhanced T2* or T2 contrast due to increased iron accumulation (7, 11, 13, 14). This raises the concern as to whether prior exposure to high levels of extracellular iron is required to generate the desired contrast on MR images. However, an early study in which conditional FHC over-expression was induced in tumor cells only after transplantation into the host mouse demonstrated enhanced R2 relaxation in rapidly proliferating FHC over-expressing tumors (Figure 1C) (8). A more recent study confirmed this finding when examining teratomae caused by uncontrolled

proliferation of FHC over-expressing murine embryonic stem cells (10). Similarly, pronounced signal voids were detected in the location of grafted skeletal myoblasts that over-expressed FHC following intramyocardial injection in mice after MI (Figure 1D) (12, 40). Interestingly, despite the lack of pre-incubation in iron supplemented medium, all three studies (8, 10, 12) demonstrated measurably enhanced $R2/R2^*$ between 14–21 days after injection. These findings are extremely promising for clinical translation of ferritin over-expression for imaging of cell survival and proliferation. Specifically, compared to labeling of cells with iron oxide nanoparticles, the contrast provided by ferritin over-expression does not diminish as a function of proliferation. In addition, the time required for ferritin over-expressing cells to accumulate sufficient iron in order to generate strong $R2/R2^*$ contrast, even under ischemic conditions, falls well within the typical time that elapses between clinical visits. While the constitutive presence of hypointensity at locations of implanted cells may limit acquisition of additional MRI measurements, this limitation could be overcome through the use of switchable reporter constructs in which ferritin over-expression can be turned on or off at will and detected within several days (8, 38). Notably, over-expression of ferritin results in suppression of the damaging Fenton reaction and thus can protect cells from oxidative damage, thereby enhancing the survival of the administered cells. These results suggest that members of the ferritin family hold significant promise as translational MRI reporter genes for *in vivo* imaging of cell survival/proliferation.

Compared to the native and the engineered ferritin constructs reported so far, iron oxide particles, due to their magnetite core and their higher iron payload, possess sufficient $R2^*$ relaxivity to enable *in vivo* MRI tracking of small numbers of cells (41). Utilizing the capacity of iron oxide particles to generate strong $T2^*$ contrast at sparse densities, several recent studies have developed MRI reporter genes that enable targeted delivery of iron oxide particles to and increased storage within select cells. In an early study by Weissleder et al., an engineered TfR (ETfR) served as a reporter protein in stably transfected gliosarcoma cells (25). Expression of ETfR alone did not alter image contrast in tumors generated using transfected gliosarcoma cells (25). However, intravenous administration of holo-transferrin covalently bound to iron oxide nanoparticles generated significant contrast in transfected gliosarcoma cells on $T2^*$ -weighted images (Figure 2A) (25). This method was later applied both *in vitro* (27) and *in vivo* (28) to imaging of therapeutic gene transfer to cancer cells. The heightened sensitivity to lower cell densities makes this technique well suited for application to imaging of cell survival and proliferation in settings where cell density is a limiting factor. Further, as SPIO particles have already seen clinical use, additional toxicological testing of holo-transferrin bound SPIO particles could be performed in a straightforward manner.

A different recently proposed reporter gene which also acts via generation of strong $T2/T2^*$ contrast, that which encodes for the protein magA, was initially associated with the production of magnetosomes in magnetotactic bacteria (42, 43). In a recent study by Zurkiya et al., doxycycline dependent magA expression in 293T mammalian cells was initiated immediately following injection of 10^5 -293T cells into the brains of mice (42). With neither prior expression of magA, nor exposure to iron supplemented medium, pronounced contrast on $T2^*$ -weighted images of the mouse brain clearly identified the population of magA expressing cells only five days after implantation and induction of magA expression (Figure 2B) (42). While these initial results are promising, the mechanism of contrast generation remains unclear, as a recent study questioned the involvement of magA in the magnetosome pathway (44). Furthermore, the bacterial origin of this reporter and the lack of a mammalian homolog raises the possibility of an immune reaction that could lead to elimination of cells expressing MagA.

In contrast to the use of iron binding proteins, an alternative approach is the use of LacZ as an MRI reporter enzyme capable of activating exogenously delivered substrate analogs to yield MRI contrast agents (15, 16, 19) for detection of cell survival and proliferation. In an elegant early study by Louie et al., a gadolinium based contrast agent termed EgadME was designed to maintain a gadolinium ion in a water inaccessible state until undergoing enzymatic reaction with β -gal (16) (Figure 3A). Following enzymatic cleavage, the gadolinium ion transitions to a water accessible state, and enables generation of strong T1 contrast on MR images (16). In this study, *Xenopus* embryos at the two-cell stage were isolated and received injections of EgadME in both cells, and a DNA construct carrying the LacZ gene in one cell (16). T1-weighted MR imaging of the same embryo at a later embryonic milestone demonstrated increased signal intensity in tissues derived from the cell injected with both EgadME and the LacZ DNA (16). The spatial distribution of LacZ expression was later confirmed with β -gal staining of the whole embryo.

In a recent study by Arena et al., a gadolinium based contrast agent activated by β -gal activity was used to image the proliferation of mouse melanoma cells (19). In order to overcome the reduction in contrast resulting from the dilution of the contrast agent with each cell division, the authors designed a novel contrast agent that, following reaction with native tyrosinase activity, underwent aggregation and further enhanced T1 relaxation (Figure 3B) (19). Following intratumoral injection of the contrast agent into both control and LacZ expressing tumors, the authors used T1-weighted MR image acquisition to demonstrate selective signal enhancement, up to several hours after injection, in LacZ expressing tumor cells (19). In a parallel attempt to overcome concerns associated with pre-labeling of cells, several recent studies have examined generation of contrast on T2* weighted images using the accumulation of chelated ferric iron from the reaction between β -gal and the commercially available staining salt S-gal (17, 18). LacZ-expressing and wild-type (WT) human breast cancer cells were injected into the flanks of mice and allowed to proliferate (17). Following intratumoral injection of a mixture of S-gal and ferric ammonium citrate (FAC), LacZ expressing tumor cells appeared hypo-intense as compared to WT tumors (Figure 3C) (17). This approach was evaluated also for skeletal muscle grafts from mice genetically engineered to over-express LacZ, implanted into the hind limb of WT mice (18). Following intramuscular injection of S-gal+FAC, the implanted cells could be clearly identified by MRI for up to 24 hours (18). Most recently, Yu et al. detailed the development of a β -gal activated, dual $^{19}\text{F}/^1\text{H}$ contrast agent (20). *In vivo* testing of the most promising candidate in a xenograft tumor model in mice demonstrated significant T2 shortening following intra-tumoral FAC injection in LacZ expressing tumors, but not in WT tumors (Figure 3D) (20).

While results from pre-clinical studies demonstrated promise in the context of spatially predefined application, the common requirement for pre-labeling of cells, or direct injection into the tissue of interest, serves as a major barrier to clinical translation. For clinical application, the LacZ substrate analog must be administered intravenously and penetrate target cells with high efficiency. A substrate with a cell-penetrating peptide was recently reported in order to enhance cellular uptake of a gadolinium based contrast agent following intravenous administration (45). Using *in vitro* preparations of rat C6-glioma cells, the authors demonstrated enhanced R1 relaxation following cellular uptake of the contrast agent in preparations of LacZ-expressing cells (45). Such a mechanism could potentially enable intravenous administration, however, the required doses would have to be large enough to overcome non-specific cellular uptake of the contrast agent en route to the tissue of interest. Furthermore, the pharmacokinetics of retention and clearance of the delivered substrate and the β -gal reaction product in all organs have not been comprehensively examined. This is a point of concern, as several of the substrates and products could have biological side effects.

Until these concerns are adequately addressed, use of LacZ as an MRI reporter gene is likely to remain confined to pre-clinical research.

An innovative alternative proposed to overcome the barrier of cellular internalization of contrast agent complexes has been to use engineered cell surface peptides as reporter proteins. For example, a recent study by Tannous et al. designed a metabolically biotinylated trans-membrane receptor to function as a reporter protein (Figure 4A) (21). MRI contrast was generated following intravenous, not intra-tissue, injection of streptavidin conjugated to either magnetic nanoparticles, or horseradish peroxidase along with a peroxidase-sensitive gadolinium compound (21). In mice bearing tumors that expressed the biotinylated receptor, intravenous injection of streptavidin-conjugated magnetic nanoparticles resulted in a dramatic decrease in the T2 of transgenic tumors compared to control tumors (Figure 4A) (21). When the authors repeated the experiment using conjugated gadolinium, T1 shortening was over three times greater in tumors expressing the biotinylated receptor as compared to controls (21). In a study by Chuang et al., expression of a plasma membrane-anchored anti-polyethylene glycol (PEG) peptide was used as a reporter protein in cancer cells, and intravenous injection of PEG-tagged SPIO particles were used for MRI contrast (22). Experiments performed *in vivo* in mice demonstrated strong labeling by PEG-tagged SPIO particles of tumors that expressed the anti-PEG reporter (22). While many studies which utilized delivery of a targeted MRI contrast agent to an engineered membrane-bound reporter protein were performed in pre-clinical cancer models where a large mass of the transgenic cells could be imaged, one recent study examined application of such techniques in the context of a pre-clinical study of stem cell therapy for cardiac regeneration. Chung et al. implanted embryonic stem cells (ESC), which had been engineered to express the reporter proteins myelocytomatosis (myc) and hemagglutinin (HA) as plasma membrane bound complexes, into infarct zone myocardium following MI in mice (23). Regions of surviving/proliferating ESC were detected on T2*-weighted images following intravenous injection of SPIO particles conjugated to either anti-myc, or anti-HA monoclonal antibodies (Figure 4B) (23). Further, by spacing follow up scans appropriately to allow for washout of labeled SPIO particles, a clear time-course of proliferation and teratoma formation was determined (23). In contrast to the previously described methods, Westmeyer et al. recently proposed a system by which the reporter peptide is secreted, instead of tethered to the plasma membrane (46). Using secreted alkaline phosphatase (SEAP) as an MRI reporter enzyme, engineered and reactive aggregates of iron oxide particles could function as exogenously delivered, activated contrast agents (46). Disaggregation of the iron oxide particle complex, which led to reduced signal intensity in T2-weighted MR images under *in vitro* conditions, was catalyzed via the hydrolysis of phosphorylated adenosine derivatives (Ado) by secreted SEAP (46). The results of these studies are highly promising as they combine the strengths of paramagnetic contrast agents with efficient and highly targeted intravenous delivery. However, it is important to note that MRI reporter genes that require secondary administration of activated contrast agents may not be appropriate for disease models in which ischemia or low vascular permeability and interstitial diffusion/convection may limit contrast agent delivery, or in the brain where contrast agent complexes may be unable to cross the blood brain barrier.

The MRI reporter gene methods hitherto discussed share two limitations relative to optical reporters: (1) low capacity for multiplexing: imaging of at most two reporter genes (one acting to modulate T1 and one to modulate T2 relaxation) is possible in one session, and (2) low dynamic resolution: lifetime of the contrast generated upon suppression of expression will depend on clearance of the reporter probe. However, many biological processes, including the differentiation of pluripotent cells, involve expression of a large number of genes of interest (47), with complex dynamic patterns of expression. With this in mind, an attractive emerging MRI reporter gene method is expression of tailored artificial peptides

making use of the chemical shift sensitivity of chemical exchange saturation transfer (CEST)-MRI (29). Unlike the aforementioned methods that use T1 or T2 shortening mechanisms to generate image contrast, CEST-MRI uses the chemical exchange of radiofrequency (RF) labeled protons between the CEST agents and nearby water molecules in order to generate contrast on MR images (Figure 5A). One particular advantage of CEST reporter genes is that compared to other methods, the lifetime of CEST contrast is determined by the T1 relaxation of intracellular water, and not by the body's clearance of labeled contrast agents. As a result, with adequate separation of the resonant frequencies of CEST reporter proteins, imaging of several CEST reporter proteins can be performed in one imaging session (Figure 5A).

The first candidates for CEST reporter genes generally demonstrated limited contrast to noise ratio (CNR). However, CNR was elegantly enhanced by Gilad et al. who constructed an artificial CEST reporter gene, the lysine-rich-protein (LRP), capable of producing robust CEST contrast (30). This was demonstrated both *in vitro*, and *in vivo* following transfection and neural implantation of glioma cells in mice (Figure 5B) (30). These findings were later expanded by McMahon et al. who developed and tested, *in vitro*, thirty three different polypeptides capable of both producing CEST contrast and functioning as reporter genes (48) (Figure 5A). This process was optimized in a subsequent study that demonstrated a high-throughput method for assessment of such CEST agents (49). More recently, CEST-MRI has been used to monitor enzymatic activity (32), as well as to measure the contrast generated by poly-L-lysine alongside T2 contrast generated by SPIO particles when mixed together in phantoms (50).

Until recently, the high susceptibility of CEST-MRI to inhomogeneities in the static magnetic field (B_0) had limited the ability to accurately detect cells which expressed CEST reporter genes. However, concomitant advancements in CEST image acquisition and post-processing methods have improved CNR and significantly reduced sensitivity to B_0 -inhomogeneities. These methods include the “water saturation shift referencing” (WASSR) (31), and “length and offset varied saturation” (LOVARS) (33) methods. A recent study which used the LOVARS method to image gliosarcoma cells (which are known to produce CEST contrast through amide proton transfer) injected into the brains of mice demonstrated improved tumor definition with enhanced CNR when compared to existing CEST-MRI methods (Figure 5C) (33). To date, *in vivo* CEST-MRI studies have been performed using constitutive expression of only one reporter protein. However, the rapid development of both CEST reporter proteins and improved CEST acquisition methods suggests that with further *in vivo* experiments, CEST-MRI could enable concomitant *in vivo* imaging of the survival and proliferation of a number of different cell types. The capacity of CEST imaging to identify a large number of MRI reporter genes in a reversible manner that does not alter underlying anatomical detail, enables additional MRI measurements, and does not require administration of exogenous contrast agents, makes this method a highly promising candidate for translational regenerative therapies. With respect to cell therapy for neurological regeneration, CEST reporters overcome many of the barriers to contrast agent delivery described previously. However, CEST methods such as the LOVARS technique would require further development before application to cardiac imaging in order to determine the effects of cardiac motion during varied saturation durations.

IV. MRI Reporter Genes for Imaging of Cell Migration

The migration of cells from remote sites, such as the recruitment of immune cells, bone marrow derived cells or circulating precursor cells, is common across diseases including stroke, cancer and infarct healing after MI. Researchers seeking to investigate the migration of specific cells frequently rely on transgenic cell lines that express fluorescent or

luminescent reporter genes. A number of MRI studies have approached the problem of tracking specific cells via *ex vivo* loading of cells with MRI contrast agents including gadolinium chelates, fluorinated emulsions, or a variety of iron oxide particles (for a full description, please consult the MR cell tracking review in this special issue). While such methods provide high sensitivity and enable MRI tracking of small numbers of labeled cells, their efficacy drops with time due to loss of the cell label with cell division (5), insufficient clearance or secondary uptake of contrast agent after cell death (51), and potential toxicity of the contrast agent. Several of the MRI reporter genes described above hold promise as alternative strategies for MRI of cell migration, however, few studies have examined the direct application of MRI reporter genes for imaging of cell migration.

Three recent studies have used ferritin over-expression in neural progenitor cells (52, 53), and in fibroblasts (54), to enable *in vivo* imaging of cell migration. In the first study, Iordanova et al. targeted native primary neural progenitors using a stereotactic injection of an adeno-virus encoding for a chimeric ferritin molecule (55) containing both FHC and FLC subunits (L*H) (52). By injection into the subventricular zone (SVZ), an area of the brain rich with neural progenitor cells, the authors were able to target expression to neural progenitors that migrate from the SVZ to the olfactory bulb (52). T2*-weighted MRI performed ten days after injection of the adeno-virus highlighted a stream of L*H expressing neuroblasts en route to the olfactory bulb in the brains of mice (Figure 6A) (52). Hypointensities which were observed on MR images demonstrated strong correlation with histological assessment of cell migration (52). While these results are promising, a highly similar study performed by Vande Velde et al. which used injection of a lenti-virus encoding for FHC detected FHC induced T2/T2* contrast only in *ex vivo*, and not *in vivo* images (53). Work from this group has previously highlighted concerns regarding both contrast generated in the immediate area of viral injection that is unrelated to induced ferritin over-expression, and significant differences in contrast generated based on the choice of viral vector (56). These findings should be kept in mind for studies that seek to label and track cells entirely *in vivo* using viral mediated selective ferritin over-expression.

In our lab, we recently used over-expression of FHC in a line of CV-1 fibroblasts to monitor fibroblast recruitment to human ovarian tumors in nude mice (54). Four days after initiation of hind-limb tumors, 2×10^6 FHC over-expressing fibroblasts that were pre-labeled with ferric citrate were administered remotely from the tumor via intraperitoneal injection. Systemic recruitment of fibroblasts was monitored using R2-mapping of the tumor at a series of subsequent time points. Sample R2-maps acquired 7 days after administration of fibroblasts (Figure 6B) demonstrate heightened R2 at the tumor rim in mice that had received FHC over-expressing fibroblasts, but not in mice that had received control fibroblasts. The results of these studies represent a promising start for the application of ferritin for *in vivo* MRI of cell migration.

Although ferritin has to date been the reporter gene of choice for *in vivo* imaging of cell migration, several of the previously mentioned reporter gene methods could also be applied. For example, imaging of engineered plasma membrane targeted reporter peptides with activateable contrast agents, or imaging of migrating transgenic cells expressing CEST reporters, could also be used as MRI reporter mechanisms for imaging of cell migration.

V. MRI Reporter Genes for Imaging of Cell Differentiation

Significant investment has been made in recent years into regenerative cell therapy for cardiovascular (57, 58) and neurological (59) diseases, and to cell based therapies of cancer (60). In most cases, undifferentiated pluripotent or multipotent cells are injected into the organ of interest, in the hope that the microenvironment at the site of injection will induce

differentiation of injected cells into the desired cell type. The role of MRI has generally been limited to assessing survival and proliferation of injected cells, with other modalities being assigned the roles of imaging cell differentiation (61). Imaging of MRI reporter genes of cell differentiation presents a more stringent set of requirements than imaging of cell survival, proliferation, and even migration. Expression of the MRI reporter gene must produce sufficient contrast to enable detection of low levels of cell differentiation at sparse densities, as has been noted in pre-clinical and early clinical trials (57, 58). Although MRI reporter genes for cell differentiation have not been examined following transplantation in the context of cell therapy, early studies using tissue specific promoters have enabled *in vivo* MRI of the differentiation of cells harboring particular MRI reporter genes. This capability was demonstrated in two studies which used either ferritin (37), or engineered plasma-membrane bound (24) MRI reporter proteins for detection of tissue specific transcription during fetal development of transgenic mice.

In an early study that examined use of ferritin over-expression for imaging of transcriptional regulation, Cohen et al. demonstrated that ferritin could be used as an MRI reporter gene for *in vivo* imaging of cell differentiation, even at low densities such as with vascular endothelial cells (37). Using double transgenic mice with endothelial cell-specific over-expression of FHC, *in utero* MRI revealed enhanced R2 in fetal organs replete with growing blood vessels, namely the heart and liver, amongst double transgenic embryos and not neighboring single transgenic control embryos (37) (Figure 7A,B). Furthermore, *in vivo* MRI of the brain of adult mice demonstrated enhanced R2 in the hippocampus region of double transgenic, but not single transgenic control mice (37) (Figure 7C-E). While this is the only instance of use of ferritin as a reporter gene of cell differentiation, the suitability of ferritin has been further affirmed by pre-clinical gene therapy trials which used ferritin over-expression to monitor gene delivery in the rodent brain (56, 62), and in models of cancer (39). Although these studies used constitutive expression of ferritin following gene delivery (39, 56, 62), they demonstrated positive results under constraints similar to those of imaging cell differentiation. Furthermore, recent studies have begun to investigate mechanisms for improving the R2 relaxivity of ferritin through genetic engineering techniques (63), or by aggregation of ferritin molecules into larger complexes (64). The promising results of early studies, as well as the potential for heightened contrast using engineered ferritin complexes, suggest that clinical translation of ferritin as an *in vivo* MRI reporter gene of differentiating pluripotent cells is possible.

Plasma membrane bound reporter proteins that can be targeted with MRI contrast agents were discussed earlier in the context of cell survival/proliferation. Recently, Bartelle et al. described a plasma membrane bound biotinylated reporter (termed the Biotag system) that could be targeted with avidinated chelated gadolinium (av-gadolinium) (24). In transgenic murine embryos that expressed the Biotag system under the control of a vascular endothelial cell promoter, injection of avidin-Gd enabled high resolution imaging of embryonic microvasculature as well as major blood vessels including cerebral blood vessels (24). In contrast, signal enhancement in WT embryos was restricted to the site of injection. Further, *in vivo* studies were performed following subcutaneous implantation of VEGF supplemented Matrigel plugs, which are known to recruit host endothelial cells as part of an induced angiogenic response (24). T1-weighted MRI demonstrated significant signal enhancement of Matrigel plugs implanted in Biotag expressing mice, but not WT mice, only 1 hour after injection of av-gadolinium via a tail vein catheter (24). Using similar principles, Suero et al. imaged the differentiation of host vascular endothelial cells participating in angiogenesis in flank tumors injected into control and Ts-Biotag (65). The authors further noted that, compared to existing biotin-avidin targeted MR imaging methods, the Biotag system resulted in greater biotin labeling of each reporter molecule, thereby significantly increasing the sensitivity to areas of sparse cell density (24). While the restricted

intravascular activity of avidinated gadolinium limits application of this technique to studying endothelial cell differentiation, it is highly relevant to a large number of developmental, disease, and regenerative models.

VI. Conclusions

The field of MRI reporter genes has experienced rapid development over the last few years. A large number of MRI reporter gene methods (Table 1) are now available for *in vivo* imaging of cell survival and proliferation in pre-clinical cancer, neurological, and cardiovascular models of disease. While only ferritin over-expression has been applied to MRI of cell proliferation, migration and differentiation, several other techniques described in this review are applicable to all three. Recent improvements in the design of novel cell surface receptors for targeted contrast media and novel CEST reporter peptides and imaging strategies suggest exciting potential for MRI reporter gene imaging. Ideally, several of the methods described in this review could be combined in one imaging session to elucidate complex multi-parametric cellular processes. Importantly, several of the MRI reporter gene methods hold strong translational potential, and could help to bridge the gap which exists between pre-clinical research using conventional reporter genes and clinical application of novel therapies.

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Abbreviations

CEST	Chemical exchange saturation transfer
FHC	Ferritin heavy chain
FLC	Ferritin light chain
β-gal	β-galactosidase
SPIO	Superparamagnetic iron oxide
Gd	Gadolinium

References

1. Stepanenko OV, Verkhusha VV, Kuznetsova IM, Uversky VN, Turoverov KK. Fluorescent proteins as biomarkers and biosensors: throwing color lights on molecular and cellular processes. *Curr Protein Pept Sci.* 2008; 9(4):338–369. [PubMed: 18691124]
2. Waidmann MS, Bleichrodt FS, Laslo T, Riedel CU. Bacterial luciferase reporters: The Swiss army knife of molecular biology. *Bioengineered Bugs.* 2011; 2(1):8–16. [PubMed: 21636983]
3. Gilad A, Ziv K, McMahon M, van Zijl P, Neeman M, Bulte J. MRI reporter genes. *J Nucl Med.* 2008; 49(12):1905–1908. [PubMed: 18997049]
4. Gilad A, Winnard PJ, van Zijl P, Bulte J. Developing MR reporter genes: promises and pitfalls. *NMR in Biomed.* 2007; 20(3):275–290.
5. Shapiro EM, Skrtic S, Koretsky AP. Sizing it up: Cellular MRI using micron-sized iron oxide particles. *Magn Reson Med.* 2005; 53(2):329–338. [PubMed: 15678543]

6. Koretsky A, Lin Y, Schorle H, Jaenisch R. Genetic control of MRI contrast by expression of the transferrin receptor. *Proc ISMRM*. 1996
7. Deans AE, Wadghiri YZ, Bernas LM, Yu X, Rutt BK, Turnbull DH. Cellular MRI contrast via coexpression of transferrin receptor and ferritin. *Magn Reson Med*. 2006; 56(1):51–59. [PubMed: 16724301]
8. Cohen B, Dafni H, Meir G, Harmelin A, Neeman M. Ferritin as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors. *Neoplasia*. 2005; 7(2):109–117. [PubMed: 15802016]
9. Genove G, DeMarco U, Xu H, Goins WF, Ahrens ET. A new transgene reporter for in vivo magnetic resonance imaging. *Nat Med*. 2005; 11(4):450–454. [PubMed: 15778721]
10. Liu J, Cheng EC, Long RC, Yang SH, Wang L, Cheng PH, Yang J, Wu D, Mao H, Chan AW. Noninvasive monitoring of embryonic stem cells in vivo with MRI transgene reporter. *Tissue Eng Part C Methods*. 2009; 15(4):739–747. [PubMed: 19290800]
11. Kim H, Cho H, Choi S, Woo J, Moon W. In vivo imaging of tumor transduced with bimodal lentiviral vector encoding human ferritin and green fluorescent protein on a 1.5T clinical magnetic resonance scanner. *Cancer Res*. 2010; 70(18):7315–7324. [PubMed: 20823165]
12. Naumova AV, Reinecke H, Yarnykh V, Deem J, Yuan C, Murry CE. Ferritin overexpression for noninvasive magnetic resonance imaging-based tracking of stem cells transplanted into the heart. *Mol Imaging Biol*. 2010; 9(4):201–210.
13. Choi SH, Cho HR, Kim HS, Kim YH, Kang KW, Kim H, Moon WK. Imaging and quantification of metastatic melanoma cells in lymph nodes with a ferritin MR reporter in living mice. *NMR Biomed*. 2012; 25(5):737–745. [PubMed: 22124937]
14. Campan M, Lionetti V, Aquaro G, Forini F, Matteucci M, Vannucci L, Chiappesi F, Di Cristofano C, Faggioni M, Maioli M, Barile L, Messina E, Lombardi M, Pucci A, Pistello M, Recchia F. Ferritin as a reporter gene for in vivo tracking of stem cells by 1.5-T cardiac MRI in a rat model of myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2011; 300(6):H2238–2250. [PubMed: 21335465]
15. Moats RA, Fraser SE, Meade TJ. A “Smart” Magnetic Resonance Imaging Agent That Reports on Specific Enzymatic Activity. *Angew Chem Int Ed*. 1997; 36(1):726–728.
16. Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. In vivo visualization of gene expression using magnetic resonance imaging. *Nat Biotechnol*. 2000; 18(3):321–325. [PubMed: 10700150]
17. Cui W, Liu L, Kodibagkar VD, Mason RP. S-Gal, a novel 1H MRI reporter for beta-galactosidase. *Magn Reson Med*. 2010; 64(1):65–71. [PubMed: 20572145]
18. Bengtsson NE, Brown G, Scott EW, Walter GA. lacZ as a genetic reporter for real-time MRI. *Magn Reson Med*. 2010; 63(3):745–753. [PubMed: 20146234]
19. Arena F, Singh JB, Gianolio E, Stefania R, Aime S. beta-Gal Gene Expression MRI Reporter in Melanoma Tumor Cells. Design, Synthesis, and in Vitro and in Vivo Testing of a Gd(III) Containing Probe Forming a High Relaxivity, Melanin-Like Structure upon beta-Gal Enzymatic Activation. *Bioconjug Chem*. 2011; 22(12):2625–2635. [PubMed: 22035020]
20. Yu J-X, Kodibagkar VD, Hallac RR, Liu L, Mason RP. Dual 19F/1H MR gene reporter molecules for in vivo detection of β -galactosidase. *Bioconjug Chem*. 2012; 23(3):596–603. [PubMed: 22352428]
21. Tannous BA, Grimm J, Perry KF, Chen JW, Weissleder R, Breakefield XO. Metabolic biotinylation of cell surface receptors for in vivo imaging. *Nat Methods*. 2006; 3(5):391–396. [PubMed: 16628210]
22. Chuang K, Wang H, Cheng T, Tzou S, Tseng W, Hung W, Tai M, Chang T, Roffler S, Cheng T. Development of a universal anti-polyethylene glycol reporter gene for noninvasive imaging of PEGylated probes. *J Nucl Med*. 2010; 51(6):933–941. [PubMed: 20484433]
23. Chung J, Kee K, Barral JK, Dash R, Kosuge H, Wang X, Weissman I, Robbins RC, Nishimura D, Quertermous T, Reijo-Pera RA, Yang PC. In vivo molecular MRI of cell survival and teratoma formation following embryonic stem cell transplantation into the injured murine myocardium. *Magn Reson Med*. 2011; 66(5):1374–1381. [PubMed: 21604295]

24. Bartelle B, Berrios-Otero C, Rodriguez J, Friedland A, Aristizabal O, Turnbull DH. Novel Genetic Approach for In Vivo Vascular Imaging in Mice. *Circ Res*. 2012; 110(7):938–947. [PubMed: 22374133]
25. Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, Basilion JP. In vivo magnetic resonance imaging of transgene expression. *Nat Med*. 2000; 6(3):351–354. [PubMed: 10700241]
26. Moore A, Josephson L, Bhorade R, Basilion J, Weissleder R. Human transferrin receptor gene as a marker gene for MR imaging. *Radiology*. 2001; 221(1):244–250. [PubMed: 11568347]
27. Ichikawa T, Högemann D, Saeki Y, Tyminski E, Terada K, Weissleder R, Chiocca EA, Basilion JP. MRI of transgene expression: correlation to therapeutic gene expression. *Neoplasia*. 2002; 4(6):523–530. [PubMed: 12407446]
28. Wang K, Shen B, Huang T, Sun X, Li W, Jin G, Li L, Bu L, Li R, Wang D, Chen X. MR reporter gene imaging of endostatin expression and therapy. *Mol Imaging Biol*. 2010; 12(5):520–529. [PubMed: 19957205]
29. Ward KM, Aletras AH, Balaban RS. A New Class of Contrast Agents for MRI Based on Proton Chemical Exchange Dependent Saturation Transfer (CEST). *J Magn Reson*. 2000; 143(1):79–87. [PubMed: 10698648]
30. Gilad AA, McMahon MT, Walczak P, Winnard PTJ, Raman V, van Laarhoven HWM, Skoglund CM, Bulte JWM, van Zijl PCM. Artificial reporter gene providing MRI contrast based on proton exchange. *Nat Biotechnol*. 2007; 25(2):217–219. [PubMed: 17259977]
31. Kim M, Gillen J, Landman BA, Zhou J, van Zijl PCM. Water saturation shift referencing (WASSR) for chemical exchange saturation transfer (CEST) experiments. *Magn Reson Med*. 2009; 61(6):1441–1450. [PubMed: 19358232]
32. Liu G, Liang Y, Bar-Shir A, Chan KWY, Galpoththawela CS, Bernard SM, Tse T, Yadav NN, Walczak P, McMahon MT, Bulte JWM, van Zijl PCM, Gilad AA. Monitoring Enzyme Activity Using a Diamagnetic Chemical Exchange Saturation Transfer Magnetic Resonance Imaging Contrast Agent. *J Am Chem Soc*. 2011; 133(41):16326–16329. [PubMed: 21919523]
33. Song X, Gilad AA, Joel S, Liu G, Bar-Shir A, Liang Y, Gorelik M, Pekar JJ, van Zijl PCM, Bulte JWM, McMahon MT. CEST phase mapping using a length and offset varied saturation (LOVARS) scheme. *Magn Reson Med*. 2012 Epub Ahead of Print.
34. Weissleder R, Simonova M, Bogdanova A, Bredow S, Enochs WS, Bogdanov AJ. MR imaging and scintigraphy of gene expression through melanin induction. *Radiology*. 1997; 204(2):425–429. [PubMed: 9240530]
35. Alfke H, Stoppler H, Nocken F, Heverhagen JT, Kleb B, Czubyko F, Klose KJ. In vitro MR imaging of regulated gene expression. *Radiology*. 2003; 228(2):488–492. [PubMed: 12801999]
36. Paproski RJ, Forbrich AE, Wachowicz K, Hitt MM, Zemp RJ. Tyrosinase as a dual reporter gene for both photoacoustic and magnetic resonance imaging. *Biomed Opt Express*. 2011; 2(4):771–780. [PubMed: 21483602]
37. Cohen B, Ziv K, Plaks V, Israely T, Kalchenko V, Harmelin A, Benjamin LE, Neeman M. MRI detection of transcriptional regulation of gene expression in transgenic mice. *Nat Med*. 2007; 13(4):498–503. [PubMed: 17351627]
38. Ziv K, Meir G, Harmelin A, Shimoni E, Klein E, Neeman M. Ferritin as a reporter gene for MRI: chronic liver over expression of H-ferritin during dietary iron supplementation and aging. *NMR in Biomed*. 2010; 23(5):523–531.
39. Aung W, Hasegawa S, Koshikawa-Yano M, Obata T, Ikehira H, Furukawa T, Aoki I, Saga T. Visualization of in vivo electroporation-mediated transgene expression in experimental tumors by optical and magnetic resonance imaging. *Gene Ther*. 2009; 16(7):830–839. [PubMed: 19458649]
40. Naumova A, Yarnykh V, Balu N, Reinecke H, Murry C, Yuan C. Quantification of MRI signal of transgenic grafts overexpressing ferritin in murine myocardial infarcts. *NMR in Biomed*. 2012 Epub Ahead of Print.
41. Shapiro EM, Sharer K, Skrtic S, Koretsky AP. In vivo detection of single cells by MRI. *Magn Reson Med*. 2006; 55(2):242–249. [PubMed: 16416426]

42. Zurkiya 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(6):1225–1231. [PubMed: 18506784]
43. Goldhawk D, Lemaire C, McCreary C, McGirr R, Dhanvantari S, Thompson R, Figueredo R, Koropatnick J, Foster P, Prato F. Magnetic resonance imaging of cells overexpressing MagA, an endogenous contrast agent for live cell imaging. *Mol Imaging*. 2009; 8(3):129–139. [PubMed: 19723470]
44. Uebe R, Henn V, Schüler D. The MagA Protein of *Magnetospirilla* Is Not Involved in Bacterial Magnetite Biomineralization. *J Bacteriol*. 2012; 194(5):1018–1023. [PubMed: 22194451]
45. Keliris A, Ziegler T, Mishra R, Pohmann R, Sauer MG, Ugurbil K, Engelmann J. Synthesis and characterization of a cell-permeable bimodal contrast agent targeting beta-galactosidase. *Bioorg Med Chem*. 2011; 19(8):2529–2540. [PubMed: 21459584]
46. Westmeyer GG, Durocher Y, Jasanoff A. A secreted enzyme reporter system for MRI. *Angew Chem Int Ed*. 2010; 49(23):3909–3911.
47. Sarkar P, Rao BM. Molecular aspects of cardiac differentiation in embryonic stem cells. *Crit Rev Biomed Eng*. 2009; 37(4–5):283–320. [PubMed: 20528729]
48. McMahon MT, Gilad AA, DeLiso MA, Berman SM, Bulte JW, van Zijl PC. New “multicolor” polypeptide diamagnetic chemical exchange saturation transfer (DIACEST) contrast agents for MRI. *Magn Reson Med*. 2008; 60(4):803–812. [PubMed: 18816830]
49. Liu G, Gilad AA, Bulte JWM, van Zijl PCM, McMahon MT. High-throughput screening of chemical exchange saturation transfer MR contrast agents. *Contrast Media & Molecular Imaging*. 2010; 5(3):162–170. [PubMed: 20586030]
50. Gilad AA, van Laarhoven HWM, McMahon MT, Walczak P, Heerschap A, Neeman M, van Zijl PCM, Bulte JWM. Feasibility of concurrent dual contrast enhancement using CEST contrast agents and superparamagnetic iron oxide particles. *Magn Reson Med*. 2009; 61(4):970–974. [PubMed: 19189296]
51. Winter E, Hogers B, van der Graaf L, Gittenberger-de Groot A, Poelmann R, van der Weerd L. Cell tracking using iron oxide fails to distinguish dead from living transplanted cells in the infarcted heart. *Mag Res Med*. 2010; 63(3):817–821.
52. Iordanova B, Ahrens ET. In vivo magnetic resonance imaging of ferritin-based reporter visualizes native neuroblast migration. *Neuroimage*. 2012; 59(2):1004–1012. [PubMed: 21939774]
53. Vande Velde G, Raman Rangarajan J, Vreys R, Guglielmetti C, Dresselaers T, Verhoye M, Van der Linden A, Debyser Z, Baekelandt V, Maes F, Himmelreich U. Quantitative evaluation of MRI-based tracking of ferritin-labeled endogenous neural stem cell progeny in rodent brain. *Neuroimage*. 2012; 62(1):367–380. [PubMed: 22677164]
54. Vandsburger MH, Cohen B, Addadi Y, Radoul M, Neeman M. Mapping the systemic recruitment of ferritin expressing fibroblasts to the angiogenic rim of ovarian tumors. *Proc ISMRM*. 2012
55. Iordanova B, Robison CS, Goins WF, Ahrens ET. Single chain ferritin chimera as an improved MRI gene reporter. *Prilozi, Contributions, Sec Biol Med Sci, MASA, XXXI, 2*. 2010; 31(2):151–155.
56. Vande Velde G, Rangarajan J, Toelen J, Dresselaers T, Ibrahimi A, Krylychkina O, Vreys R, Van der Linden A, Maes F, Debyser Z, Himmelreich U, Baekelandt V. Evaluation of the specificity and sensitivity of ferritin as an MRI reporter gene in the mouse brain using lentiviral and adeno-associated viral vectors. *Gene Ther*. 2011; 18(6):594–605. [PubMed: 21346786]
57. Malliaras K, Kreke M, Marban E. The Stuttering Progress of Cell Therapy for Heart Disease. *Clin Pharmacol Ther*. 2011; 90(4):532–541. [PubMed: 21900888]
58. Penn MS, Dong F, Klein S, Mayorga ME. Stem Cells for Myocardial Regeneration. *Clin Pharmacol Ther*. 2011; 90(4):499–501. [PubMed: 21934722]
59. Martino G, Pluchino S, Bonfanti L, Schwartz M. Brain Regeneration in Physiology and Pathology: The Immune Signature Driving Therapeutic Plasticity of Neural Stem Cells. *Physiol Rev*. 2011; 91(4):1281–1304. [PubMed: 22013212]
60. Al Khabori M, de Almeida JR, Guyatt GH, Kuruvilla J, Crump M. Autologous Stem Cell Transplantation in Follicular Lymphoma: a Systematic Review and Meta-analysis. *J Nat Cancer Inst*. 2012; 104(1):18–28. [PubMed: 22190633]

61. Nguyen PK, Lan F, Wang Y, Wu JC. Imaging: guiding the clinical translation of cardiac stem cell therapy. *Circ Res.* 2011; 109(8):962–979. [PubMed: 21960727]
62. Kaikkonen M, Lesch H, Pikkarainen J, Rätty J, Vuorio T, Huhtala T, Taavitsainen M, Laitinen T, Tuunanen P, Gröhn O, Närvänen A, Airene K, Ylä-Herttuala S. (Strept)avidin-displaying lentiviruses as versatile tools for targeting and dual imaging of gene delivery. *Gene Ther.* 2009; 16(7):894–904. [PubMed: 19440224]
63. Iordanova B, Robison CS, Ahrens ET. Design and characterization of a chimeric ferritin with enhanced iron loading and transverse NMR relaxation rate. *J Biol Inorg Chem.* 2010; 15(6):957–965. [PubMed: 20401622]
64. Bennett KM, Shapiro EM, Sotak CH, Koretsky AP. Controlled aggregation of ferritin to modulate MRI relaxivity. *Biophys J.* 2008; 95(1):342–351. [PubMed: 18326661]
65. Suero Abreu GA, Bartelle BB, Aristizábal O, Houston EJ, Turnbull DH. Multimodal Molecular Imaging of Angiogenesis in a Mouse Model of Melanoma. *Proc ISMRM.* 2011

Figure 1. Iron binding MRI reporter genes for imaging of cell survival and proliferation
 (A) Representative schematic of a cell and the iron binding proteins most commonly used as MRI reporter proteins. Iron is transported into cells from holo-transferrin through the transferrin receptor (TfR), after which it is stored by ferritin. Over-expression of components of cellular iron storage enables MRI detection by increasing cellular iron content and altering T2 relaxation. Changes in the expression of iron binding proteins in subsequent representative studies are demonstrated using modifications to this schematic.
 (B) Constitutive over-expression of both ferritin heavy chain (FHC) and the TfR (top) in mouse neural stem cells enabled visual detection of only 2500 cells on T2*-weighted images following implantation into the mouse brain (bottom). The diagram on the left illustrates the locations of the cortex (ctx), striatum (stm), and injection site (is) (This material is reproduced with permission from John Wiley & Sons, Inc. and Deans et al. (7)).
 (C) Over-expression of FHC (top) in C6-glioma cancer cells resulted in significantly increased tumor R_2 as compared to control tumors (bottom) (This material is reproduced with permission from Neoplasia and Cohen et al. (8)).
 (D) Implantation of C2C12 skeletal myoblast cells

into the infarct zone of the mouse heart following MI resulted in a noticeable signal void at the site of cell proliferation only in FHC over-expressing cells (same mechanism as in C) (This material is reproduced with permission from John Wiley & Sons, Inc. and Naumova et al. (12)). Tissue staining of histological sections correlated the location of the signal void with that of grafted FHC over-expressing cells.

Figure 2. Mechanisms of enhanced T2/T2* contrast using MRI reporter genes for imaging of cell survival and proliferation

(A) Targeted delivery of holo-transferrin conjugated monocrystalline iron oxide nanoparticles (Tf-MION) to an engineered transferrin receptor (ETfR) lacking the iron regulatory element (reporter protein) enabled rapid cellular accumulation of MION particles (top). Constitutive expression of ETfR in flank tumors (white arrows) in mice did not induce image contrast (bottom left) in the absence of holo-transferrin-MION. However, administration of Tf-MION results in significant signal loss on T2 and T2* weighted images (bottom middle) in ETfR expressing tumors (left tumor). The change from baseline R2 was significant in ETfR tumors (bottom right) (This material is reproduced with permission from Nature Publishing Group and Weissleder et al. (25)). (B) MagA, a protein adapted from magnetotactic bacteria for use as an MRI reporter gene, uses endogenous iron to produce magnetic nanoparticles within magA expressing cells. Injection of magA expressing cells into the brains of mice results in pronounced signal attenuation on T2*-weighted images as early as five days after injection (top, arrow), resulting from the high density of produced magnetic nanoparticles (bottom) (This material is reproduced with permission from John Wiley & Sons, Inc. and Zurkiya et al. (42)).

Figure 3. Expression of β -galactosidase as an MRI reporter enzyme for imaging of cell survival and proliferation

β -galactosidase (β -gal), an inveterate reporter enzyme in biomedical research, is traditionally used in combination with histological techniques. Recently, a number of studies have sought to use β -gal as an enzyme for activation of exogenously delivered MRI contrast agents capable of producing contrast via a range of mechanisms. (A) EgadME, an engineered gadolinium based contrast agent (top) maintains the gadolinium ion in a water inaccessible state until activation by β -gal expression. *Xenopus* embryos were injected with EgadME at the two-cell stage, but only the descendents of the single cell that expressed LacZ (the gene which encodes for β -gal) demonstrate increased signal intensity on T1-weighted MR images (middle) and whole embryo β -gal staining (bottom) acquired at a later embryonic milestone (This material is reproduced with permission from John Wiley & Sons, Inc. and Louie et al. (16)). (B) A gadolinium based contrast agent is activated by β -gal activity, and further aggregates to enhance T1-relaxation upon reaction with native tyrosinase activity (top). Application *in vivo* in cancer cells (white circles) implanted in the flank of a mouse demonstrated prolonged signal enhancement on T1-weighted images only in tumors expressing LacZ (bottom) (Reprinted (adapted) with permission from Arena et al. Bioconjugate Chemistry. 2011;22:2625–2635. (19)). (C) Borrowing directly from histological staining techniques, the interaction between β -gal and a staining salt (top) in the presence of ferric ammonium citrate (FAC) results in chelated ferric iron that produces a dark stain in histology, and T2* contrast in the presence of LacZ expressing cells. Images acquired prior to (bottom left) and after (bottom right) intratumoral injection of the staining salt S-gal and FAC demonstrate hypo-intensity only in the LacZ expressing tumor (This material is reproduced with permission from John Wiley & Sons, Inc. and Cui et al. (17)). (D) A ^{19}F -labeled contrast agent that, upon reaction with β -gal, chelates iron and produces

T2 contrast on ^1H -MR images (top). Intratumoral injection of this agent led to significant T2 shortening in LacZ expressing (purple arrows), but not control tumors in mice (bottom) (Reprinted (adapted) with permission from Yu et al. *Bioconjugate Chemistry*. 2012. 23(3): 596–603. (20)).

Figure 4. Targeted MR imaging of cell surface expressing reporter proteins

(A) Constitutive expression of a biotinylated transmembrane receptor (BAP-TM) as a reporter protein (left) enabled *in vivo* imaging of gene expression using streptavidin conjugated to either magnetic nanoparticles (right) or peroxidase sensitive gadolinium contrast agents (bottom). Following injection of magnetic nanoparticle conjugated streptavidin, tumors expressing BAP-TM demonstrate significantly reduced T2 values (right) (21). In contrast, administration of peroxidase sensitive gadolinium and streptavidin results in hyper-intensity in BAP-TM expressing tumors on T1 weighted images (bottom, red arrows) (21)(Reprinted with permission from the Nature Publishing Group and Tannous et al. (21)). (B) Embryonic stem cells (ESC) engineered to express membrane bound HA and myc antigens as reporters (top) were implanted into the infarct zone of the mouse heart following myocardial infarction (bottom). T2*-weighted MR imaging of the heart after intravenous administration of a targeted - iron oxide nanoparticle conjugated - contrast agent demonstrated signal voids in the presence of proliferating, and teratoma forming, ESC (bottom) (This material is reproduced with permission from John Wiley & Sons, Inc. and Chung et al. (23)).

Figure 5. CEST MRI of reporter peptides

(A) CEST-MRI uses the exchange of radio frequency labeling from the protons of the CEST contrast agent to those of nearby water molecules (left, grey arrow) (Reprinted with permission from the Nature Publishing Group and Gilad et al. (30)). The saturation of spins resonating at the CEST agent frequency (green bar at NH peak in middle), after allowing for exchange, results in a decrease in the magnitude of the water peak (30). (Right) Polypeptides with strong CEST potential were dissolved in phosphate buffered saline (PBS) (magnitude) and imaged using three different saturation frequencies (This material is reproduced with permission from John Wiley & Sons, Inc. and McMahon et al. (48)). A merged image of magnetization transfer maps, normalized to maximum signal intensity and color coded based on saturation frequency, illustrates the ability to image several unique CEST labels for several polypeptide reporters (DIACEST) (48). (B) An engineered CEST reporter peptide with enhanced amide magnetization transfer, termed the lysine rich protein (LRP) was transfected into glioma cells prior to implantation in the mouse brain (30). Anatomical reference images (top) illustrate similar signal intensity in LRP and control xenografts (30). In contrast, the map of changes in signal intensity in CEST experiments highlights the location of LRP expressing xenografts (Reprinted with permission from the Nature Publishing Group and Gilad et al. (30)). (C) Improved pulse sequence and post processing techniques have led to enhanced sensitivity and accuracy of CEST-MRI. (i) T2-weighted scout image demonstrates signal enhancement at the site of a gliosarcoma tumor in the mouse brain. Gliosarcoma cells are known to demonstrate CEST contrast through amide (NH) proton transfer. (ii) B₀ shift map (acquired using the WASSR technique (31)) illustrates the range of B₀ inhomogeneity across the mouse brain. (iii) Conventional measurement of magnetization transfer ratio (MTR) illustrates the effect of B₀ inhomogeneities on the accuracy of conventional CEST-MRI. (iv) Implementation of the LOVARS method, represented by a threshold LOVARS imaginary component map super-

imposed on a magnitude reconstructed reference image, ameliorates the error introduced by B_0 inhomogeneities and enables accurate *in vivo* MRI of CEST reporters (This material is reproduced with permission from John Wiley & Sons, Inc. and Song et al. (33)).

Figure 6. In vivo imaging of cell migration using ferritin as an MRI reporter gene
 (A) Injection of an adenovirus into the sub-ventricular zone (SVZ) of the mouse brain generated a population of ferritin heavy chain (FHC) and ferritin light chain (FLC) over-expressing neural progenitor cells (L*H). Migration of L*H neuroblasts from the SVZ to the olfactory bulb (white arrows) was successfully imaged using T2*-weighted MRI. The asterisk (*) in images indicates the injection sites of control (top asterisk) and L*H-adenovirus (bottom asterisk) (Reprinted with permission from Elsevier and Iordanova et al. (52)).
 (B) R2 mapping of human ovarian cancer tumors in the hind limbs of mice 7 days after intraperitoneal injection of either control (top) or FHC over-expressing fibroblasts (bottom). Preferential recruitment of fibroblasts to the tumor rim is visualized as significantly elevated R2 values in mice receiving FHC over-expressing (bottom), but not control (top) fibroblasts (54).

Figure 7. MRI reporter gene imaging of endothelial cell differentiation

Use of double transgenic mice, in which tTA mediated ferritin over-expression was regulated by transcription of the vascular endothelial (VE) cadherin promoter, enabled *in utero* and *in vivo* detection of endothelial cell differentiation (37). (a) Representative *in utero* MRI of single transgenic (tTA-Ferritin, left) and double transgenic (VE-cadherin-Ferritin, right) embryos demonstrated enhanced R_2 in the fetal heart (H) and liver (L), as well as in the placenta (P), in the double transgenic embryo (37). (b) At embryonic day 13.5 (E13.5), elevated expression of VE-cadherin in differentiating endothelial cells in the liver and heart resulted in significantly elevated R_2 measurements in double transgenic (middle, green) as compared to single transgenic (middle, gray) embryos (37). When the change in R_2 over the brain, which demonstrates low VE-cadherin activity at E13.5, was measured, single and double transgenic embryos could be distinctly identified. (c) In the adult mouse, expression of ferritin in vascular endothelial cells was visualized as elevated R_2 values in the brain. R_2 values measured in the cortex (C), and in the hippocampus (H) were elevated in double transgenic mice (e) as compared to single transgenic mice (d). In the hippocampus, the increase in R_2 was significant in all slices through the brain (Reprinted with permission from the Nature Publishing Group and Cohen et al. (37)).

Table 1

Classification of MRI reporter gene methods in the context of imaging of cell survival/proliferation, migration, and differentiation.

	Mechanism of Contrast	Cell Survival and Proliferation	Cell Migration	Cell Differentiation
Ferritin	T2/T2*	Cohen et al. (8) Campan et al. (14) Kim et al. (11) Liu et al. (10) Deans et al. (7) Choi et al. (13) Naumova et al. (12, 40)	Iordanova et al. (52) Vande Velde et al. (53) Vandsburger et al. (54)	Cohen et al. (37)
β -gal + Activated Contrast Agent	T1/T2/T2*	Moats et al. (15) Louie et al. (16) Cui et al. (17) Arena et al. (19) Bengtsson et al. (18) Yu et al. (20)	Louie et al. (16)	
Tyrosinase	T1	Weissleder et al. (34) Paproski et al. (36) Alfke et al. (35)		
magA	T2/T2*	Zurkiya et al. (42)		
Engineered Transferrin Receptors (ETfR)	T2/T2*	Weissleder et al. (25) Moore et al. (26) Ichikawa et al. (27) Wang et al. (28)		
Plasma membrane bound reporter peptides	T1/T2/T2*	Tannous et al. (21) Chuang et al. (22) Chung et al. (23) Bartelle et al. (24)		Bartelle et al. (24) Suero et al. (65)
CEST	CEST	Gilad et al. (30) Liu et al. (32) Kim et al. (31) Song et al. (33)		

Studies are sorted based on the *in vivo* models that were used.