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Reporter gene approaches for mapping cell fate decisions by MRI: promises and pitfalls

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The central dogma of molecular biology, namely the process by which information encoded in the DNA serves as the template for transcriptional activation of specific mRNA resulting in temporal and spatial control of the translation of specific proteins, stands at the basis of normal and pathological cellular processes. Serving as the primary mechanism linking genotype to phenotype, it is clearly of significant interest for *in vivo* imaging. While classically, imaging revolutionized the ability to phenotype the anatomical and physiological impact of induction of changes in gene expression, the preceding molecular events remained invisible. Reporter gene-based imaging techniques provide a window for *in vivo* visualization of such transcriptional activation events. In addition to the widespread use of fluorescent and bioluminescent reporter genes and development of a number of reporter genes for positron emission tomography (PET) imaging, there has been significant progress in the development of reporter genes for MRI. With the development of strategies for cellular based therapies, such imaging tools could become central components for personalized patient monitoring. © 2013 The Authors. *Contrast Media & Molecular Imaging* published by John Wiley & Sons, Ltd.

Keywords: reporter genes; molecular imaging; validation

1. INTRODUCTION TO REPORTER GENES

Survival, proliferation, migration, differentiation and even death of cells are executed by timely induction or suppression of the transcription of specific genes that generate mRNA, which is subsequently translated to form proteins such as enzymes, cytoskeleton proteins, signaling molecules, receptors and growth factors. A large fraction of the genome is devoted to regulating the timely control of transcription, through specific regulatory elements, which bind transcription activators or suppressors. Such molecular control of genetic programs includes, for example, the response to hypoxia, mediated by stabilization of Hif1 allowing its interaction with the hypoxia response element, found in HIF1 target genes such as VEGF (1). Detection of the activity of such regulatory response elements can be done by direct analysis of the expression of the target genes. However, such analysis is often invasive, and in addition, it is affected by confounding effects of additional regulatory layers such as the control of translation and stability (2). For example, intracellular iron homeostasis is controlled both by transcriptional and by translational regulation via the activity of the antioxidant response element in the DNA promoter and iron response element on the mRNA, which regulates the intracellular level of transferrin receptor, ferritin and other target genes (3).

Visualization of the translation control itself can be isolated using an artificial gene in which the genomic promoter of interest drives the expression of an easily detectable reporter. Thus one of the important strengths of reporter genes lies in their capability to reveal well-defined molecular steps in gene expression. Beyond the ability to probe the activity of endogenous modulators of transcription, reporter genes can be constructed with tailored promoter regulatory sequences that allow sculpting their temporal and tissue specificity (4). Such design is key for the application of reporter genes for cell lineage tracing and cell tracking (Figure 1).

2. TYPES OF MRI REPORTER GENES AVAILABLE

2.1. Iron Homeostasis Proteins

The first reporter gene proposed for MRI by Koretsky *et al*. was the transferrin receptor (5). Overexpression of transferrin receptor was expected to raise the level of labile iron in the cells, and thus lead to storage of that excess iron within ferritin. While overexpression of transferrin receptor yields only a weak change in image contrast, the abundance of transferrin receptors can be detected at high sensitivity through administration of transferrin conjugated to iron oxide nanoparticles, thus facilitating detection of the reporter gene, by using a matching reporter probe (6–8). One possible drawback of this approach is the potential for augmenting Fenton reaction owing to the elevated level of intracellular free iron.

Ferritin, the ubiquitous iron storage protein, is probably the most popular reporter gene for MRI (9–33). Overexpression of the heavy chain of ferritin alone, or in combination with the light chain of ferritin or with the transferrin receptor, results in elevation of intracellular ferritin iron stores, and a corresponding

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change in R_2 relaxation. As for endogenous ferritin in ferritin-rich organs such as the spleen, the relaxivity per iron of the ferrihydrite core within ferritin is low as compared with that induced by magnetite, but detectable contrast can be achieved with a physiological level of expression.

Although both transferrin receptor overexpression and ferritin overexpression ultimately result in elevated intracellular iron stores, the impact on iron homeostasis is opposite. Overexpression of ferritin results in attenuation of the intracellular labile iron pool and thus reduces the damaging effects of iron in catalysis of

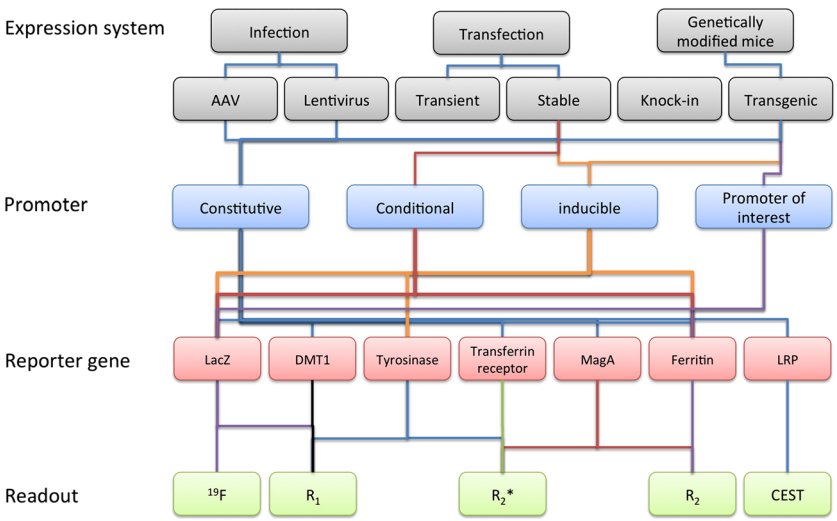

Figure 1. Schematic representation of the possibilities for reporter gene applications for MRI. Experimental setup requires multiple tiers of design, each of which can significantly affect the sensitivity and specificity for detection. In particular, the expression system, the promoter of interest, the reporter gene itself and the MRI pulse sequence design should be optimized, and should include built-in readout for validation of the acquired data. Colored connections were demonstrated to be feasible resulting in detectable MRI contrast.

the Fenton reaction. The rise in iron stores is due to compensatory elevation in iron uptake by the cells so as to maintain iron homeostasis. Thus transferrin receptor overexpression activates an iron overload response, whereas ferritin overexpression activates an iron deficiency cellular response.

MagA, an iron transporter from magnetotactic bacteria, was evaluated as a reporter gene for MRI (32,34). Similar to overexpression of transferrin receptor, magA overexpression resulted in increased R_2 relaxation and detectable change in contrast.

2.2. Reporter Enzymes

The divalent metal transporter, DMT1, was suggested as a possible reporter gene, allowing enhanced manganese uptake in DMT1 overexpressing cells (35). In contrast to the signal attenuation of the iron-based reporter genes, uptake of manganese results in signal enhancement that can be detected with high sensitivity, albeit at the potential cost of systemic exposure to manganese toxicity.

Tyrosinase was suggested as a reporter gene for MRI already in 1997 by Weissleder *et al.* (36). Tyrosinase is the key enzyme in melanin synthesis and thus overexpression of tyrosinase results in accumulation of melanin, which sequesters paramagnetic ions and thus generates MR contrast. Tetracycline-regulated expression of tyrosinase resulted in corresponding alteration in MR contrast *in vitro* (37). Recently, the interest in tyrosinase was renewed with its application as a multimodal reporter which can be detected not only by MRI but also by photoacoustic imaging and by PET (38,39).

The lacZ gene, which encodes for the expression of β-galactosidase, is a widely used reporter gene that is usually used for *ex vivo* analysis of gene expression in histological specimens. Being an enzyme with no endogenous contrast, the β-galactosidase activity resulting from lacZ reporter gene expression is detected with the use of analog substrates that function as reporter probes. Reporter probes for detection of galactosidase activity by MRI have also been developed (40–45). Recently an elegant probe was reported by Arena *et al.*, which allowed conditional detection of β-galactosidase activity specifically in tyrosinase expressing cells (46). Enzymatic cleavage of the probe by β-galactosidase releases a Gd–DOTA containing substrate for tyrosinase, thus leading to incorporation of the contrast media in melanin.

2.3. Synthetic Reporters with Endogenous Contrast

Chemical exchange saturation transfer (CEST) MRI is sensitive for the presence of amide protons that exchange with the bulk water. Such exchange can be used for generation of chemical shift selective contrast in the absence of paramagnetic species. Gilad *et al.* constructed an artificial lysine rich protein (LRP), which can be expressed by cells resulting in detectable MRI contrast (47). Detection of this reporter depends on selective RF irradiation of the exchanging protons and thus can be 'switched' on or off at will, depending on the MRI pulse sequence applied. Recently, the same group constructed an elegant reporter gene, which functions as a substrate analog for protein kinase A, revealing its enzymatic activity in phosphorylation (48).

2.4. Methods for Inducing Expression of Reporter Genes

2.4.1. Transfection

Expression of a transgene in cells is achieved by inducing uptake of the relevant DNA plasmid into the cells across the cell membrane. This can be achieved with the aid of calcium phosphate, cationic lipid transfection agents, nanoparticles, electroporation, with the use of ultrasound or with the use of membrane permeating peptides. In such transfection the DNA is usually not integrated into the cell genome, resulting in transient expression. By addition of a resistance gene followed by selection, it is possible to isolate stably transfected cells in which expression remains constant with cell proliferation; however, there could still be variance in the level of expression owing to differences in the number of copies of the transgene and their integration site. Such stably transfected cells can be used for isolation of individual clones, which can be expanded in order to achieve a uniform expression level in all cells.

It is important to note here that caution must be used in attributing a change of phenotype to the expressed transgene, as phenotype could be affected also by the site of integration and by the selection of a specific clone.

2.4.2. Infection

Reporter genes can be incorporated in gene delivery systems such as *viral vectors* for transduction of cells. Viral vectors make use of the naturally evolved infection properties of the viruses they are derived from to efficiently shuttle foreign pieces of genetic material into target cells. Viral vectors are engineered in such a way that the genetic information for replication of the virus in the target cell is not present in the vector, so that no secondary infection or disease can occur upon reporter gene delivery. For this reason, this process is often referred to as 'transduction' to differentiate it from infection by the native virus.

There are viral vectors systems available for (reporter) gene delivery based on adenoviruses (AV), retroviruses (RV) including lentiviruses (LV) such as HIV-1, adeno-associated viruses, poxviruses and herpes simplex viruses (HSV) (49). These vector systems all have different properties (i.e. possible insert size, target cell tropism, transient or stable transgene expression, immunogenicity, safety, production titer, shelf life, etc.), mainly depending on the virus they are derived from [for a more detailed overview, see Merten *et al.* (49)]. Depending on the desired gene transfer and target cell population of the respective application, an appropriate viral vector system has to be used.

Adenoviral vectors will transduce both actively dividing as quiescent cells and have a pronounced liver tropism after systemic administration. This is illustrated by their usefulness for targeting mitotically active cancer cells as well as for nondividing liver or brain cells (50–52). Adeno-associated viral vectors also efficiently transduce dividing and nondividing cells and have a broad target cell tropism depending on the (recombinant) capsid (53). The advantage of Adeno-associated viral vectors is that high levels of gene expression can be reached that are stable over the long-term (years) in different types of nondividing cells and tissues including the brain (25,54,55), but there is a smaller size limit on the transgene(s) that can be shuttled (53). Where a larger insert size needs to be accommodated, retroviral vectors (RV) can be considered as a transduction vehicle. Because of the ability of reverse transcription of their single-stranded RNA genome into dsDNA that is then integrated in the target cell genome, RVs are capable of stably transducing target cells and their progeny, which is a major advantage for studies where long-term labeling

is desirable. However, the reverse transcription step makes RVs incompatible with synthetic reporter genes comprising repeated sequences, such as, for example, the artificial LRP reporter gene proposed for CEST-based MRI (47). RVs based on simple retroviruses are only capable of transducing actively dividing cells. Therefore, they can be used to selectively label mitotically active cell populations where the label needs to be stably transmitted to the daughter cell populations (56). On the other hand, complex retroviruses like lentiviruses such as HIV-1, are able to also infect nondividing cells. Lentiviral vectors derived from HIV-1 are very useful for long-term gene transfer into mature cell types, as has been demonstrated for neurons in the brain (25,57). As HSV is a naturally neurotropic virus, viral vectors based on HSV-1 can also be used for gene transfer applications in the brain (58).

Most viral vectors offer the possibility of multicistronic gene expression, depending on the maximum possible insert size. The expression of multiple reporter genes is important in the perspective of multi-modality imaging in order to overcome the limitations of individual imaging modalities. Many potential applications would benefit from regulable or inducible promoter systems (e.g. tet-on, tet-off). Viral vector-mediated gene transfer is perfectly compatible with inducible promoter systems. Apart from tight regulation of reporter gene expression, the incorporation of suicide genes is possible in viral vectors so that derailed genetically altered cells can be ablated with prodrugs that are harmless for nontransduced cells (e.g. HSV-tk).

2.4.3. *Transgenic animals*

A reporter gene can be expressed by genetically modified mice using two primary strategies. The transgene can be injected into early stage mouse embryos. The mice are then selected for germ cell transmission of the transgene to their progeny. Alternatively, the reporter gene DNA can be selectively incorporated into the correct location in embryonic stem cells, and these cells are then used for generation of knock-in mice, in which the reporter gene expression is regulated by the endogenous promoter of interest. Over recent years, selective control of reporter gene expression is achieved through mating of mice with conditional expression of the reporter gene, with generic 'driver' mice which can induce the relevant induction of expression through tissue-specific expression of cre recombinase, or by specific treatment with an antibiotic (e.g. tetracycline) or a drug (e.g. tamoxyfen) (4). The main strength of these conditional inducible systems is their ability to direct the expression at will to highlight the process of interest. As such, tetracycline-inducible expression of ferritin was used for temporal and tissue specific detection of reporter gene expression in transgenic mice by MRI (22,59).

2.5. Pitfalls in use of Reporter Genes

2.5.1. *Integration site and copy number*

The site of transgene insertion in the genome and the number of transgene copies can significantly impact the level of expression and the ability of the transgene to respond to the regulatory cues of interest. Even worse, however, is the possibility that the incorporation of the transgene will alter the function of an endogenous gene at the site of integration and thus alter phenotype, or even lead to malignant transformation. Thus it is critical to verify that the phenotype of the cell (or the transgenic mouse) is not altered by the reporter gene. Through the use of Flp recombinase, it is possible to direct the transgene to the specific location of interest in the host genome (60,61).

2.5.2. *Fidelity of promoter constructs*

Regulatory elements controlling the expression of a gene of interest can be found at a significant distance from the coding area. Thus, a transgene that includes a putative promoter sequence linked to a reporter gene may not accurately report the regulation of expression of the endogenous gene. This is particularly true for genes whose expression could be regulated by a complex integrated input from multiple regulatory sequences. Direct insertion of the reporter gene into the endogenous gene could provide a more accurate record of the regulation of the gene expression. However, it is also important to note that the expression of the endogenous gene could be altered by such manipulation, and this could result in a specific possibly unwanted phenotype, particularly for those genes in which gene dosage is important for their function.

2.5.3. *Tissue specificity*

Apart from the target cell tropism determined by the (recombinant) vector system used, reporter gene expression in specific target cells can be achieved using cell- or tissue-type-specific promoters. However, their use often results in lower expression levels as compared with constitutive promoters. Therefore, incorporating reporter genes by Cre/*loxP*-mediated recombination in transgene animals expressing Cre recombinase in specific cells or tissues can be a preferred alternative.

2.5.4. *Temporal resolution*

In the case of genetic labeling, incorporation in an inducible expression system is possible, which holds the potential for dynamic sensing of gene expression under the condition that the contrast can be cleared by normal cellular pathways to restore the signal to baseline when it needs to be in the 'off' position. While iron from overexpressed ferritin is metabolized through the tightly regulated physiological iron metabolism pathways, the dynamics for generation and loss of contrast relative to induction of gene expression remain to be studied. The temporal resolution achieved by reporter genes is confounded by the time for translation of the protein, the lifetime of the reporter gene mRNA and the reporter protein, and the time for delivery of the reporter probe and clearance of the reporter product. In most cases the temporal resolution is expected to be on the order of hours to days, and thus this approach is usually limited for detection of slow processes.

2.5.5. *Specificity/ background issues*

Depending on the viral system used, vector-based delivery of reporter genes can elicit nonspecific contrast owing to the vector itself at the site of injection that is detected by MRI (25,62). In the case of direct *in vivo* transduction with MRI reporter genes, this background contrast can be a consequence of an immune response to the viral vector. As cells of the immune system are also able to accumulate paramagnetic ions like iron, a misleading hypointense contrast in T_2*-weighted MR images can be a consequence (25). However, this confounding effect occurs at very

small distances, so that tracking of cells migrating away from the site of labeling remains possible (63). The much smaller extent of image distortion in the case of genetic labeling with MR reporters as compared with labeling with paramagnetic particles would still allow targeting of embryonic neuronal precursor cells (eNPC) migration over much smaller distances, such as in the dentate gyrus of the hippocampus.

2.6. Validation

Dual expression of two reporter genes (e.g. ferritin and luciferase or GFP) can be used for validating one reporter relative to the other *in vivo* or by immunohistochemistry (25,57,63). Such an approach is particularly helpful when the two reporters and their detection by the corresponding image modality differ in sensitivity, *in vivo* depth of penetration and/or the availability of anatomical data. It is important to make sure in preparation of the construct that expression of the two reporter genes is linked (57), or to prepare the two reporters as a single chimeric protein to form a multimodality reporter gene. It is always useful to add to the reporter genes small epitope tags for IHC (10), as histology often remains the gold standard for validation purposes. In the case of viral vectors, the transduction of a cell can be validated by PCR of vector-specific sequences or in situ hybridization (ISH) against integrated vector sequences in the case of retrovirus (RVs) and lentivirus (LVs). When using a specific promoter of interest for driving the expression of the reporter gene, it is important to validate the fidelity of the promoter by independent analysis of the expression of the endogenous gene of interest.

3. APPLICATIONS

3.1. Neurogenesis

In neurogenesis imaging, cells to be visualized longitudinally include neural stem cells and their progeny which are actively dividing cells. For such applications, genetically labeling these cells with a reporter gene has a clear advantage over particle-based labeling methods as a genetic label integrated in the cell genome would be readily transmitted to the progeny, whereas a particle label would become diluted with every cell division. Additionally, the expression of a reporter gene can be made dependent on the differentiation status of a cell. Therefore, the use of several reporter genes and delivery systems for cell tracking using different imaging modalities has been explored for neurogenesis imaging. Once the genetic label has been stably integrated in the host cell genome through transgenesis or stable vector transduction, potential dilution of contrast agents and subsequent signal loss upon cell division of stem and progenitor cells is not a concern. For example, the use of a neuronal precursor-specific, that is, the double-cortin promoter to drive luciferase expression, enabled bioluminescence imaging (BLI) of neural progenitor cell proliferation and migration (64).

Alternatively, viral vectors can be used to stably label stem and/or progenitor cell populations by topical injection. A good example here is the differential labeling of the different neurogenic populations in the brain. Using the capability of retroviral vectors to stably transduce mitotically active cells, they have been used for differential labeling of neural progenitor cells in the subventricular zone, providing a readout on neuroblast proliferation (56). As lentiviral vectors can stably transduce both dividing and nondividing cells, injection in the subventricular zone results in efficient marking of the slowly dividing type B stem cells and rapidly dividing progenitor cells, with gene expression passed on to the stem cell progeny, allowing tracking of eNPCs during proliferation, migration and differentiation using sensitive immunohistochemistry for eGFP (65,66), but also *in vivo* using BLI (67).

Whereas BLI is to be considered as mainly a 2D imaging method with only limited tomographic potential, MRI has a long track record as imaging modality enabling stem cell tracking in 3D with excellent soft tissue contrast, which makes it perfect for imaging the brain (68). Since the proof of concept for ferritin as a genetic cell marker for *in vivo* MRI (10,69), the feasibility of using ferritin as an MRI reporter by *in situ* viral vector-mediated delivery in the brain has been shown for different viral vector systems (25,28,52). Using lentiviral vectors expressing ferritin to label the eNSCs, the stem cell progeny migrated and integrated in the olfactory bulb (OB) could be detected and quantified with *ex vivo* MRI, but for *in vivo* tracking of the migrating stem cell progeny, the system lacks sensitivity (63). In search of improving the paramagnetic properties of ferritin, linking the light and heavy chains of ferritin resulted in a slightly larger protein cage and improved MR contrast *in vitro* (17). Using an adenoviral vector encoding this chimeric ferritin reporter to label eNPCs, migrating cells could be visualized along the rostral migratory stream (RMS) with *in vivo* MRI at 11.7 T (62). However, no contrast was seen in the OB where these cells arrive and integrate, which could be partially due to the vector type used and/or the still rather poor sensitivity of the contrast evoked by this reporter gene.

The combined advantages of MRI for high-resolution brain imaging and the cell specificity of genetic labeling will remain a driving force in the search for more sensitive genetic MRI reporter systems for neurogenesis imaging. Although most genetic approaches to increase cellular MR contrast are based on iron sequestrating proteins (15,70–72), any adverse influence on iron homeostasis in the brain must be carefully evaluated because of its role in neurodegenerative processes (73,74).

3.2. Cardiac Imaging

Very few studies have exploited so far the available MRI reporter genes for cardiac imaging. In a set of elegant studies, Naumova *et al*. followed ferritin overexpressing mouse skeletal myoblasts transplanted in the infarcted mouse heart, demonstrating a 20–30% reduction in signal intensity using two pulse sequences: bright-blood *T*(2)-weighted gradient echo (GRE) and black-blood *T*(2)-weighted GRE with improved motion-sensitized-driven equilibrium preparation (21,27). These studies highlight the importance of optimizing the acquisition scheme in order to reliably detect contrast changes. In another study, cardiac stem cells derived from swine were engineered to express human ferritin heavy chain. These cells could be detected by 1.5 T clinical MRI after their administration to infarcted rat hearts (23).

3.3. Cancer

Progression of tumors is a multistep process. Beyond the malignantly transformed cancer cells, it also involves a large number of accessory host cells, which although genetically normal, are altered phenotypically in the tumor microenvironment and contribute significantly to tumor progression. As a pathological

process with extensive dynamic remodeling, reporter genes can contribute significantly to our knowledge about the critical steps of cancer progression.

Multiple studies have applied MRI reporter genes for the study of cancer. In most of these, the reporter gene was expressed under a constitutive promoter by the cancer cells themselves, allowing tracking of tumor cell proliferation, without dilution of the label as seen for exogenous labeling (e.g. using iron oxide particles). Moreover, the contrast detected could be attributed directly to viable cancer cells without the confounding effect of secondary uptake of contrast probes by macrophages. In some of the studies a switchable promoter was used. Reporter gene-based MRI was also applied for monitoring recruitment of stroma cells into the tumor (33).

In a first example, tetracycline inducible expression of the heavy chain of ferritin was used for detection of tetracycline induced MRI contrast changes. Tumor xenografts of C6 glioma showed enhanced R_2 relaxation rate, which was reduced in tumors in which overexpression of h-ferritin was suppressed by addition of tetracycline to the drinking water (10). A similar approach by another group demonstrated MRI detectable changes using tetracycline-regulated expression of ferritin in nasopharyngeal carcinoma (26). In another glioma study, 9L glioma cells expressing an artificial LRP were inoculated to the brain of mice, showing a CEST MRI signal which was specific for the LRP-expressing tumor (47). Alternatively, mouse GL261 glioma cells were induced to express a chimeric ferritin-DsRed fusion protein, serving as a dual modality reporter gene for MRI and fluorescence imaging (16). In another example, a chimeric ferritin with enhanced iron binding, R_2 and R_2* relaxivity was applied for detection of transgene expression in human osteosarcoma cells (17). Green fluorescent protein (GFP) and myc-tagged human ferritin heavy chain expressed in tumor xenografts were used for demonstration of the ability to detect the reporter gene even when using a 1.5 T clinical MRI system (19). Colonization of tumors with probiotic *Escherichia coli* bacteria expressing bacterioferritin could be detected by MRI though changes in R_2 contrast (24). Recently, ferritin-expressing stroma fibroblasts were applied for tracking their systemic recruitment to remote human ovarian carcinoma xenografts in mice (33).

Also transporters of metals were used as reporter genes in models of cancer. MagA, the iron transporter from magnetotactic bacteria, was overexpressed in mouse neuroblastoma N2A cells, resulting in increased iron uptake and MRI detectable contrast (34). The contrast generated by MagA in tumors was comparable to that generated by overexpression of ferritin (32). The divalent metal transporter DMT1 was applied for inducing manganese uptake into cancer cells, thus resulting in R_1-based contrast (35).

A novel probe for ^{19}F MR, 2-fluoro-4-nitrophenyl β-D-galactopyranoside, showing distinct change in chemical shift after its hydrolysis by β-galactosidase, was used for differentiating wild-type from lacZ-expressing prostate tumor xenografts in mice (43). A bimodal cell permeable substrate for β-galactosidase, Gd–DOTA–k(FR)–Gal–CPP, was developed for MR and fluorescence detection of lacZ in C6 tumors (42). Melanoma cells typically express the melanin-forming enzyme tyrosinase. A Gd-based substrate of tyrosinase, which is activated by β-galactosidase, provides a novel tool for selective detection of reporter gene expression by the melanoma cells (46). Tyrosinase itself was expressed as a reporter gene for MRI in MCF7 human breast cancer cells as a reporter gene for MRI and photoacoustic imaging (38) and recently also for PET (39).

4. SUMMARY

Reporter genes open up novel possibilities for *in vivo* cellular and molecular imaging in different domains such as neurogenesis, cardiac and cancer research. Using constitutively activated promoters, reporter genes can be used for tracking cell migration and following expansion in a number of administered cells. In these applications, reporter genes overcome major limitations of specificity and dilution by proliferation which limit the interpretation of data acquired with cells labeled using exogenous probes such as dyes or nanoparticles. In addition, reporter genes open up novel possibilities for noninvasive visualization of transcriptional and translation regulation of genes. Such applications can be used for visualization of programmed differentiation pathways in cells. Clearly this latter possibility could have profound implications in regenerative cellular therapy. Administration of genetically modified cells must always be performed with care, and indeed, the use of reporter genes can be subject to multiple confounding effects associated with the mode of transduction, epigenetic and selective effects, and complex regulatory mechanisms that can impact the expression level *in vivo*. However these same considerations also apply to any therapeutic genetic modification, and thus with careful design, reporter genes for *in vivo* imaging could provide a much needed companion diagnostic readout for monitoring such interventions.

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