# Review of MRI Reporter Genes in Oncology

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**Abstract.** Molecular imaging aims to detect molecular events in entire organisms. Even though it is not possible to directly detect the genes and proteins located in intracellular compartments, it is possible to detect them via reporter genes that encode for the specific proteins. The reporter gene has gained importance in diagnostics, enabling monitoring of gene expression patterns and in vivo monitoring of cell survival, proliferation, migration and differentiation. As cancer is the one of the most significant burdens for both population health and healthcare systems, it is of high importance to devote significant efforts towards timely diagnosis. The MRI reporter genes can be used for this purpose. For the purposes of this work, the following databases were searched: ScienceDirect, PubMed and Google Scholar. The inclusion criteria is the publications containing keywords such as MRI, MRI reporter gene, MRI reporter gene cancer, Molecular-Genetic Imaging, and reporter gene imaging, language of publication is English, articles including *in vivo* monitoring released between 2000 and 2023, and full-text journal publications, conference publications, standards, guidelines, and books. Applying the previous criterion 24 articles were found and analyzed. This review will present some of the MRI reporter genes that are used in oncology with an emphasis on most recent trends.

**Keywords:** MRI reporter genes · Oncology · Cancer

## 1 Introduction

Magnetic Resonance Imaging (MRI), a non-invasive imaging technology, produces three dimensional detailed anatomical images. It is frequently used for disease detection, diagnosis, and treatment monitoring [1]. The sensitivity and specificity of MRI was increased by the addition of reporter genes. When introduced into target cells - brain tissues, cancer and circulating white cells, reporter genes produce a protein receptor or enzyme that binds, transports or traps a subsequently injected imaging probe. Reporter genes imaging is an effective tool for studying molecular activities in living organisms. MRI reporter genes are genes that encode for proteins that can be imaged using magnetic resonance imaging (MRI) techniques. These genes have the potential to be powerful tools for non-invasive monitoring of gene expression and cell tracking in living organisms. In oncology, MRI reporter genes are being developed to improve the detection and treatment of cancer [2].


A. Badnjević and L. Gurbeta Pokvić (Eds.): MEDICON 2023/CMBEBIH 2023, IFMBE Proceedings 93, pp. 263–269, 2024.
https://doi.org/10.1007/978-3-031-49062-0_28

The first MRI reporter gene utilized in the past was creatine kinase (CK), an enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) while simultaneously producing phosphocreatine (PCr). This process can be detected by $^{31}$P Magnetic Resonance Spectroscopy (MRS) [3]. Reporter genes for MRI may be used to precisely track the cell delivery in cell therapy, analyze the therapy effect of gene delivery, and monitor tissue/cell-specific microenvironments. By visualizing the levels of exogenous or endogenous gene expression, specific signal transduction pathways, nuclear receptor activities, or protein–protein interactions, MRI reporter gene imaging can longitudinally monitor the processes (e.g., cell delivery, gene expression, et al.) in living organisms [4, 5]. In order to measure expressed reporter gene protein, various detection methods are used. These methods include luminescence, absorbance and fluorescence [6]. Commonly used reporter genes for MRI usually include genes encoding the enzyme (e.g., tyrosinase and β-galactosidase), the receptor on the cells (e.g., transferrin receptor), and endogenous reporter genes (e.g., ferritin reporter gene) [7–9].

MRI can visualize functional physiologic changes or biochemical changes, as well as anatomic morphologic changes using radiolabeled metabolic substances, such as glucose, amino acids, or nucleotides.

Classified MRI approaches on the basis of interactions at the molecular level: the expression of surface receptors that allow the binding of specific MRI contrast agents; the enzyme-based cleavage of functional groups that block water (proton) exchange, protein binding, or MRI contrast agents; and the expression of para- and antiferromagnetic proteins involved with iron metabolism, such as tyrosinase and ferritin [10].

Despite the potential of MRI reporter genes, there are still obstacles to their broad usage. One significant problem is getting high levels of reporter gene expression in target cells, which is required for precise imaging. Moreover, the sensitivity of MRI methods may restrict the capacity to identify modest levels of gene expression.

Finally, MRI reporter genes have the potential to be useful tools for tracking cancer progression and therapy. Aim of this paper is to present all MRI reporter genes which are used in oncology with the emphasis on most recent trends. Further study is needed, however, to improve their expression and imaging capabilities, as well as to establish their therapeutic value. Therefore, the purpose of our review is to present the current state of research on MRI reporter genes in oncology, so it can help to advance the field and identify areas for further study.

## 2 Methodology

ScienceDirect, PubMed and Google Scholar were reviewed to find as much information as possible about reporter genes in oncology analyses. The following search criteria were used to limit the research:

1) publications containing keywords: Magnetic Resonance Imaging, MRI reporter gene, MRI reporter gene cancer, Molecular-Genetic Imaging, and Reporter gene imaging
2) language of publication: English
3) publication time: 2000–2023
4) full-text journal publications, conference publications, standards, guidelines, books.

A summary of the most commonly studied genes that have been used as MRI reporters in oncology is presented in this paper. There are two categories of reporter genes: those that can be visualized without the need to administer a substrate in the form of a contrast agent and those that rely on substrate injection to provide contrast.

## 3 Results and Discussion

By conducting a thorough examination of scientific literature and scrutinizing various methodologies for creating Magnetic Resonance Imaging (MRI) reporters, three distinct contrast mechanisms/agents were identified, namely T2 or T2*, T1, Chemical Exchange Saturation Transfer (CEST). This review comprehensively presents the reporters and sensors utilizing the above-mentioned contrast mechanisms and elaborates on their individual features. After the screening process, 10 reporter genes were found to be most suitable for the intended purpose.

In a study by Qin et al. MCF-7-TYR human breast cancer cells have been transfected with a plasmid encoding tyrosine (TYR) and untransfected MCF-7 cells served as the study's negative controls. MCF-7-TYR tumors achieved much larger signals and tumor-to-background contrasts than MCF-7 tumors in *in vivo* MRI imaging studies, among others such as PET and PAI [11]. The finding that increased levels of tyrosinase activity in melanotic melanomas led to stronger signal intensity in T1-weighted MR images of patients led Vandsburger et al. to investigate whether overexpressing tyrosinase could be used as a way to track cell survival through MRI [12]. Initial studies showed that introducing human tyrosinase to murine fibroblasts and human kidney cells resulted in higher MRI signal intensity. Two subsequent studies by Alfke et al. and Paproski et al. confirmed these findings by inducing the expression of tyrosinase in human breast cancer cells [13, 14].

Arena et al. used the *Lac Z* gene as an MRI reporter to track mouse melanoma cell proliferation by administering a gadolinium-based contrast agent that is cleaved by the β-galactosidase enzyme expressed by *Lac Z*. Cancer cells expressing *Lac Z* show higher contrast on T1 weighted MRI compared to control [15]. Additionally, in the study by Cui et al., the interaction between β-gal and a staining salt generates strong hypointensity on T2* weighted images in LacZ-expressing tumor cells in the presence of ferric ions [16].

According the studies by Zhou et al. from 2020 and Yang et al. from 2016, that we analyzed, ferritin heavy chain (Fth) is a promising magnetic resonance (MR) reporter gene for imaging tumors due to its ability to reduce signal intensity without fading over time and minimal cellular toxicity [17, 18]. Fth overexpression induces iron deficiency mechanisms, such as the expression of transferrin receptor (TfR), which makes it a good MR endogenous contrast agent. Moreover, Fth overexpression can enhance site-specific drug delivery by inducing the expression of TfR, which is overexpressed on cancer cells compared to normal cells [19]. Liposomes are a widely studied carrier that can be modified with transferrin (Tf) to achieve targeted drug delivery to cancer cells overexpressing TfR. Encapsulation of the broad-spectrum antitumor drug within Tf-modified liposomes (Tf-LPD) can improve therapeutic efficacy while reducing adverse effects [20].

A study by Sun et al. from 2021 aimed to develop an MRI monitoring system for early detection of potential malignant transformation of stem cells. The system required

incorporation of an MRI reporter gene and its expression in a tumor-specific manner. Fth1 was identified as an ideal MRI reporter gene, but its continuous expression made it unsuitable for detecting malignant transformation. The study modified Fth1 by adding the tumor-specific promoter PEG3 upstream, and successfully transferred it into stem cells using a recombinant lentivirus. The modified gene remained silent in normal stem cells but turned on when stem cells transformed into tumor cells, enabling MRI detection. This system could detect unexpected tumorigenicity in stem cell-based therapies and monitor the elimination of malignantly transformed cells in combination with a therapeutic gene [21]. Several studies conducted on cancerous rodent models employed Fth overexpression as a means to visualize the proliferation of murine melanoma cells upon implantation in close proximity to significant lymph nodes [22]. Additionally, Fth overexpression was utilized to monitor the proliferation of human breast cancer and rat glioma cells following subdermal implantation in mice and rats [23].

Bartelle et al. developed a system in which a cluster of biotins is displayed on cell membranes by a synthetic "Biotag" reporter. The transgene is entirely self-contained and can be employed as a vascular reporter system with any endothelial promoter, in contrast to earlier biotinylation methods. Transgenic *Ts-Biotag* mice were generated with a minimal reporter for *Tie2*. The potential of this technique for vascular imaging of genetic processes in mice was shown by the in vivo imaging of *Tie2* expression in Ts-Biotag mice, in both embryonic and adult models of vascular development [24]. Another study by Xavierselvan et al. demonstrated that imaging of tumor vasculature can be a valuable tool for preclinical cancer research. Ergo, the Biotag reporter could be used and tested for vascular MRI imaging of tumors [25].

The bacterial gene MagA can make mammalian cells magnetic and suitable for tracking through magnetic resonance imaging (MRI). In a mouse model, MagA-expressing tumors showed increased and higher quality MR contrast compared to tumors expressing a modified ferritin system lacking iron response element regulation. The results suggest that MagA expression can be used for monitoring cell growth and differentiation with the potential for *in vivo* detection of reporter gene expression using MRI [26].

The divalent metal ion transporter (DMT1) and a biotinylated cell surface protein (BAP-TM) have shown the best contrast *in vivo* among positive contrast agents for T1-weighted MRI. DMT1 led to a 75% increase in image contrast in glioma tumors, but with significant enhancement in surrounding normal tissue and variation in persistence of enhancement between tumor types [27].

b-Galactosidase is particularly useful as an MRI reporter gene because of its low background level. The presence of galactosidase will cleave the galactose and release the $Gd^{31}$ contrast agent [28]. Furthermore, the nonmetallic, biodegradable MRI reporter gene encoding lysine-rich protein (LPR) reduces MRI signal intensity through the rapid transfer of amide proton in LRP to a water proton, which produces a saturation transfer contrast in solution. The different signal intensity change for an LRP-expressing xenograft in comparison to a control xenograft in mice confirmed their potential as a reporter [4]. A study by Farrar et al. showed that incorporation of the LRP reporter gene into G47Δ, an oncolytic virus originating from herpes simplex, had no effect on the viral replication or therapeutic effectiveness. These findings are significant because they

suggest that the LRP gene can potentially be used as a reporter for the real-time detection of viral spread [29]. Tyrosinase and ferritin are metalloprotein-based MRI reporter genes, the former playing a role in melanin biosynthesis, resulting in changes in MRI signals in melanocytes and melanoma cells [13, 30]. The latter's iron-sequestering properties make it a promising MRI reporter. Cells transfected with a ferritin reporter gene have the ability to overexpress it, causing the capture of extracellular/endogenous iron, and formation of superparamagnetic crystalline iron, which leads to an observable MRI contrast [31].

Nystrom et al. have developed an imaging system using an organic anion-transporting polypeptide 1b3 (oatp1b3) as an MRI reporter gene that enables sensitive three-dimensional detection of viable cancer cells in live mice, providing high-resolution imaging and longitudinal tracking of metastatic spread to multiple lymph nodes and different organ systems in individual animals. This system could help improve our understanding of metastasis and aid in the development of new cancer therapies [32, 33].

MRI reporter genes and their details are shown in Table 1.

**Table 1.** Short review on MRI reporter genes in oncology

| Reporter gene | Contrast mechanism | Substrate | Cancer type | References |
|---|---|---|---|---|
| Tyrosinase | T2/T1 | Endogenous iron | Human breast cancer | [11, 12] |
| Ferritin | T2 | No substrate | Liver cancer; Malignant stem cells | [17, 18, 21] |
| Transferrin receptor | T2 | Tf-MIONs | Solid tumor | [19] |
| *Lac Z* | T1/T2* | S-Gal | Human breast cancer; Mouse melanoma | [15, 16] |
| β-galactosidase | T1 | Gadolinium ion | Human breast cancer; Mouse melanoma | [15, 16, 28] |
| Biotag | T1 | Gadolinium based substrate | Tumor vasculature | [24, 25] |
| MagA | T2 | Iron supplement | Melanoma | [26] |
| DMT1 | T1 | Manganese-based substrate | Melanoma | [27] |
| LRP | CEST | N/A | Rat glioma | [29] |
| Oatp1b3 | T1 | Gadoxetate disodium | Prostate cancer | [32, 33] |

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