Reporter Genes

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TARGET AUDIENCE:

Researchers and clinicians who are interested in molecular and cellular MRI, particularly in the non-invasive monitoring of gene expression, cell therapy, and transplantation, as well as pre-clinical drug screening, with advanced MRI-based techniques.

OBJECTIVES:

- ☑ To understand the need and role of MRI reporter genes in biomedicine.
- ☑ To learn the basics of gene expression in living systems.
- ☑ To review and discuss the different options for the MRI reporter genes available today.

PURPOSE

With more than 20,000 genes in the human genome now identified, and a similar number of genes in the rat and mouse genome known[1], the elucidation of their function has become the major challenge. For example, with respect to cancer, it is important to monitor the genes that escaped cellular regulation, such as oncogenes and tumor suppressor genes that can cause malignancy. Another target is to identify genes that are expressed specifically by tumors, both by the cancer cells or other cells that support the tumor, e.g., endothelial cells or cancer-associated fibroblasts[2]. These genes can serve as a diagnostic marker of the condition of the tumor or as a target for treatment, or, more relevant for our course, for imaging.

During the last two decades, reporter genes have been developed for almost every imaging modality,[3,4]. That includes optical reporter genes, such as fluorescent and bioluminescent proteins that can be detected *in vivo* with charge-coupled device (CCD) cameras. Nuclear imaging reporters are usually enzymes that are involved in the retention of radio-labeled compounds, which can be subsequently detected using a gamma camera and PET [5].

METHODS

Traditionally, measuring gene expression at the transcriptional level (RNA), translational level (protein), or post-translational level (protein degradation) involve the extraction of tissue or cultured cells. However, to obtain temporal and spatial information in real-time or non-invasively, a different approach should be taken. One such approach is to use so-called reporter genes. A reporter gene encodes a product that can be easily monitored and which is most commonly cloned, or fused directly to the gene of interest[3,4,6]. Reporter genes are usually cloned in the context of a promoter/enhancer DNA region that will allow regulated expression of these proteins in specific cells or under specific stimuli. Alternatively, reporters can be cloned as a fusion protein with a protein of interest[3,4].

The methodology is usually composed of the following steps:

- ☑ Selecting the appropriate reporter gene for the task.
- ☑ Cloning the reporter gene into the appropriate expression vector.
- ☑ Transferring the gene to the target tissue, e.g., trasnfecting/transducing cells in culture; viral or non-viral gene delivery to a live animal or generating transgenic mouse.

- ☑ Imaging the subject with the appropriate pulse sequences (T1, T2, CEST or spectroscopy).
- ☑ Image processing and data analysis.

RESULTS DISCUSSION

The principle behind genetically encoded reporters and sensors is that a new, foreign genetic material is introduced into the cell genome (either in a stable or transient mode) and utilizes the cellular transcription and translation machinery to produce a new protein, peptide, or enzyme that can affect the MRI contrast in a manner that will allow its detection with MRI. Several landmark studies have utilized genetically encoded proteins as MRI reporters, among these the human transferrin receptor[7-9], β-galactosidase[10], and proteins that are involved in iron metabolism[8,11] and storage,[12-15].

Many of these systems have already been described in previous reviews[4,16-18]. In this class, we will discuss many of these reporters, as well as newer reporters, and we will divide them into several different categories. The first category would be the contrast mechanisms via the reporter acts, this could be T1, T2/T2*, CEST, Dynamic Nuclear Polarization (DNP), 31P-, or 19F-MR. Next, the genes could be divided based on the need for substrate injection. Finally, the genes could be separated based on those that encode to reporters and those that encode to sensors. Although there is quite a bit of overlap between the two, reporters usually will provide direct information on the gene of interest or the target cells, while sensors will provide information on intra/extracellular processes or the levels of ion or metabolites.

CONCLUSION

With the more than 20 different reporter genes proposed in the past 25 years, scientists today have a battery of reporters to choose from. By understanding the features of the imaging target, it is practical today to select an optimal reporter gene based on the study goal, or even, in many cases, to design a reporter for a specific scientific question.

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