Reporter genes

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One of the challenges of imaging is to reveal tissue features beyond what can be detected using endogenous contrast mechanisms. One of the common ways to achieve that is through the use of contrast media and contrast probes, which can be directed towards specific targets, or can be loaded into cells to enable their tracking. However, often targets of interest cannot be revealed by this manner. In particular, key checkpoints such as the control of gene expression, which dictate cell behavior and determine central processes such as physiological and pathological development, cannot be revealed using the generic exogenously administered contrast media.

Reporter genes code for proteins that are readily detectable by imaging. When expressed in vivo, for example by generating transgenic cells or organisms or by viral infection, these genes serve to highlight specific intracellular expression processes. Fluorescent proteins such as the green fluorescent protein GFP, or bioluminescent enzymes such as the firefly luciferase, are widely used in biological research. Expression of these reporter genes is typically driven either by a constitutive promoter such as CMV or by inducible promoter that can be triggered on and off at will either reversibly such as the tetracycline or tamoxifen regulated promoters for transient monitoring of gene activation, or irreversibly such as the cre-recombinase for mapping cell lineages.

MRI, despite its rich capabilities for endogenous contrast, is not easily adapted for reporter gene imaging, due to its low sensitivity. However over the last years, a large number of reporter genes were suggested for MRI. Through their mode of activity, these can be divided into 5 main groups: receptors, transporters, enzymes, storage proteins and artificial proteins.

Cell surface receptors and transporters such as magA from magnetotactic bacteria, leads to accumulation of intracellular iron upon their over expression. Over expression of the ubiquitous transferrin receptor can also enhance intracellular iron, but can also be used for internalization of contrast media conjugated to transferrin. Semi-quantitative determination of the contrast changes by R_1 or R_2* relaxometry could be directed to the level of gene expression. Other transporters such as OATP1 were recently proposed for reporter gene imaging.

Enzymes such as cytosine deaminase and thymidine kinase can be detected through their activity on substrate analogues that can be detected for example by CEST imaging. Alternatively, an enzyme such as tyrosinase generates endogenous contrast upon its expression through the deposition of melanin.

Storage proteins such as ferritin is possibly the most widely used MRI reporter gene. As an iron storage protein, when over expression, it induces compensatory iron uptake so as to generate a change in field dependent R_2 relaxation.

Artificial proteins with endogenous MRI contrast were also designed, such as the lysine rich protein (LRP) that can be detected by CEST imaging.

Another way to group the reporter genes in between those that require administration of an exogenous reporter probes, such as the most of the receptors, transporters and enzymes, and those reporter genes with endogenous contrast, such as LRP and ferritin.

There are many advantages for the use of reporter genes, in which the cells are harnessed with the ability to generate their own contrast. Such label is not diluted with cell proliferation, and can be tracked over long periods. However, this technology is not immune to pitfalls, which must be considered in the design of such studies:

First and foremost, the reporter gene should be benign, it should not interfere with tissue homeostasis, and it should not alter the course of biological and pathological processes. Using a reporter gene for visualization of promoter activity depends on the fidelity of the promoter. It is often difficult to take into account the modulatory effects of remote enhancers on gene expression.

An alternative is to 'knock in' the reporter gene into the endogenous gene of interest. However, for some genes, dosage is important, resulting in detectable biological phenotype due to the loss of one allele. Additional pitfall includes the generation of contrast as an artifact associated with the delivery of the gene, eg the damage of vasculature along the needle track, and the effect associated with the site of the reporter gene integration in the genome. For reporter genes requiring delivery of reporter probes, the generated contrast can be modulated by the pharmacokinetics of the probe.

References

Cohen, B., et al. (2005). "Ferritin as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors." Neoplasia **7**(2): 109-117.

Genove, G., et al. (2005). "A new transgene reporter for in vivo magnetic resonance imaging." Nat Med **11**(4): 450-454.

Deans, A., et al. (2006). "Cellular MRI contrast via coexpression of transferrin receptor and ferritin." Magn Reson Med **56**(1): 51-59.

Cohen, B., et al. (2007). "MRI detection of transcriptional regulation of gene expression in transgenic mice." Nat Med **13**(4): 498-503.

Vande Velde, G., et al. (2011). "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 **18**(6): 594-605.

Iordanova, B., et al. (2013). "Quantification of HSV-1-mediated expression of the ferritin MRI reporter in the mouse brain." Gene Ther **20**(6): 589-596.

Iordanova, B., et al. (2013). "Engineered mitochondrial ferritin as a magnetic resonance imaging reporter in mouse olfactory epithelium." PLoS One **8**(8): e72720.

Vandsburger, M. H., et al. (2013). "Ovarian carcinoma: quantitative biexponential MR imaging relaxometry reveals the dynamic recruitment of ferritin-expressing fibroblasts to the angiogenic rim of tumors." Radiology **268**(3): 790-801.

Vande Velde, G., et al. (2013). "Reporter gene approaches for mapping cell fate decisions by MRI: promises and pitfalls." Contrast Media Mol Imaging **8**(6): 424-431.

Patrick, P. S., et al. (2014). "Dual-modality gene reporter for in vivo imaging." Proc Natl Acad Sci U S A **111**(1): 415-420.