A Bright Multi-Imaging Modality Gene Reporter

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Introduction

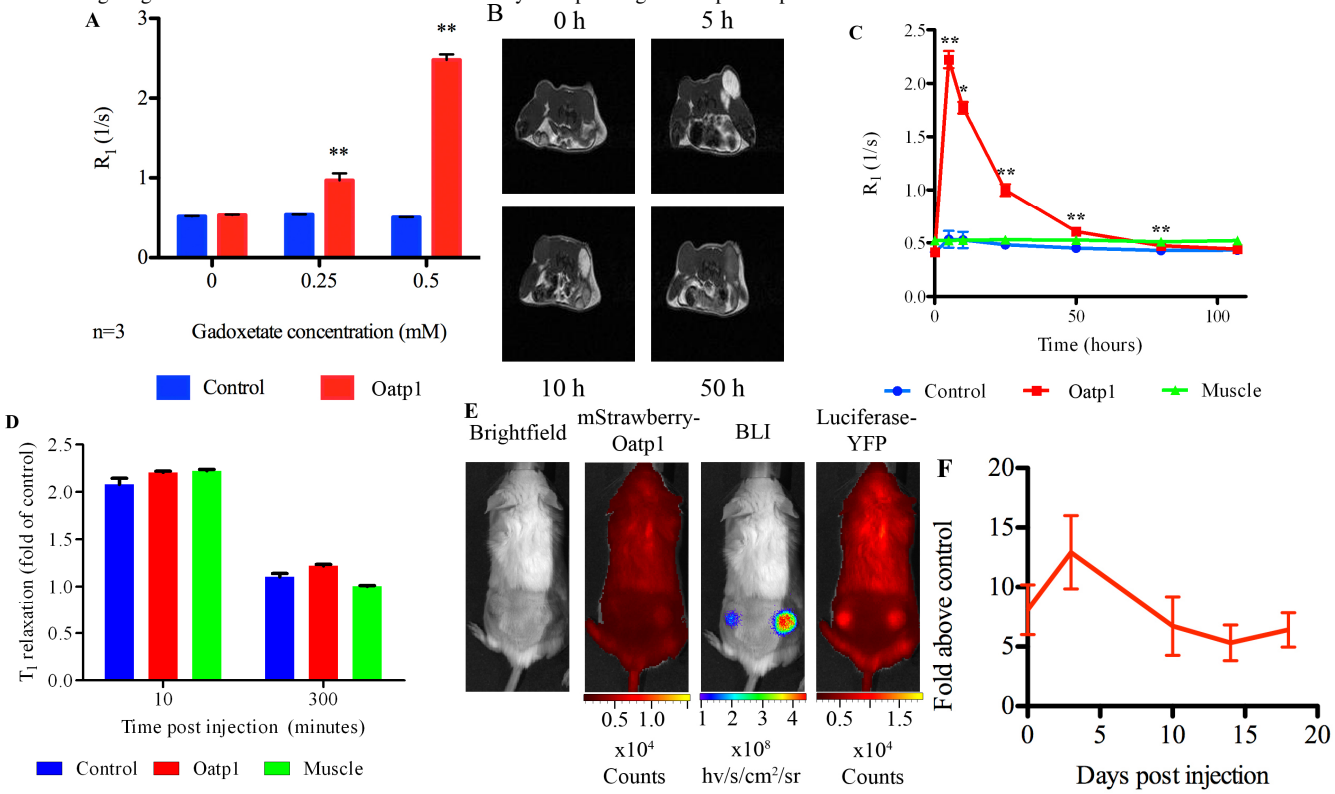
The ability to visualize and track patterns of gene expression non-invasively in living organisms could greatly enhance our understanding of tissue development and the changes in gene expression that accompany disease. Several gene reporters for bioluminescent, fluorescent, radionuclide and magnetic resonance imaging (MRI) have been described but these suffer variously from limited depth penetration, spatial and temporal resolution, and sensitivity. We describe here a gene reporter construct that could be used with any of these imaging modalities, thus combining their different strengths; demonstrating its use here with MR and bioluminescent imaging *in vivo*.

Methods and Results

The organic anion transporting polypeptide Oatp1 has been shown to transport gadoxetate - a Gd^{3+}-based hepatocyte-specific MRI contrast agent[1]. Lentivirus carrying the Oatp1 transgene together with the red fluorescent protein mStrawberry was used to transduce a clonal luciferase-expressing HEK 293T cell line. An Oatp1 sub-clone, and the cells from which this was derived (control), were incubated with gadoxetate (90 minutes), washed, pelleted, and imaged, see figure A. Oatp1-expressing cells had significantly increased R_1, while the R_1 of control cells was unaltered. To test this system *in vivo*, $1x10^7$ each of the Oatp1-expressing sub-clone, and control cells, were implanted subcutaneously into the right and left flanks of mice, respectively. Following formation of tumors and injection of gadoxetate (0.664 mmoles/ kg), R_1 significantly increased by over 4-fold in Oatp1 expressing tumors, but not control tumors, see figures B and C. Contrast enhancement was reversible and returned to background levels after 4 days. A control contrast agent (Gd-DTPA) which is not a substrate of Oatp1 was then administered at an equal dose, and contrast enhancement was observed in both tumors, followed by a rapid washout and return to background levels after 5 hours, see figure D. This demonstrated that neither tumor had non-specific contrast agent retaining properties, such as might be caused by necrosis. The ability to image gene expression longitudinally was then confirmed by subsequent gadoxetate administration, imaging, and detection of contrast in the Oatp1-expressing tumors. Furthermore we demonstrated that Oatp1 also mediates transport of D-luciferin (the substrate for luciferase), and that its expression increases bioluminescent output of luciferase-expressing cells by several-fold. This was confirmed *in vivo* using bioluminescent imaging with the same mice already described above. Oatp1 expressing tumors emitted over 5-fold more light than control tumors (both expressing luciferase) over 20 minutes post luciferin injection (150mg/kg, i.p.), see figures E and F.

Conclusions.

These results show that Oatp1 can function as an effective reporter gene for MRI, providing rapid, intense, and reversible T_1 contrast following injection of gadoxetate, with no significant background contrast in control tissue. Additionally, Oatp1 expression can increase the rate of light output of luciferase by transporting its substrate luciferin. Furthermore, the gadolinium in gadoxetate could be replaced with either ^{111}In or ^{68}Ga for SPECT or PET imaging[2], and there is evidence that Oatp1 can also mediate the uptake of dyes for fluorescence imaging. Since Gd^{3+}-based hepatocyte-specific contrast agents have been approved for human use[1], this could facilitate transfer of this technology to the clinic in the longer term. The reporter may be particularly useful for tracking implanted stem cells, since reporter gene detection should be unaffected by cell division or exocytosis, which in the case of SPIO-based labeling causes dilution of the label, and also for interrogating the differentiation state of the stem cells by incorporating tissue-specific promoters into the construct[3].



A. R_1 of cell pellets following incubation with gadoxetate. Error bars show SEM. TR= 4.22ms, TE = 1.96ms, flip angle = 90°. Scan time ~14 minutes. ** p<0.01, 2-tailed T test. **B.** T_1-weighted images of mice, before and after injection of gadoxetate. Slice thickness 1.5mm, echo time 8ms, repetition time 400ms, 50x50 mm^2 field of view, 256x128 resolution, 4 averages. **C.** R_1 of muscle, Oatp1-expressing, and control tumours, before and after gadoxetate injection. Error bars show SEM, n=5. *p<0.001, ** p< 0.0001, 2-tailed paired T test **D.** R_1 of muscle, Oatp1-expressing, and control tumours, increases above control (pre-injection R_1) at 10 minutes post injection of control contrast agent. Contrast returned to background levels after 5 hours (2-tailed paired T-test, n=2). **E.** Fluorescence imaging confirms equal expression levels of the luciferase-YFP (Yellow fluorescent protein) fusion protein in both flanks, Oatp1-expression only in the right flank, and higher bioluminescent output in the tumour expressing Oatp1. **F.** Light output of Oatp1 cells, above control cells, at various time points post implantation. On each time point light was measured every minute between 5 and 20 minutes post luciferin injection. Error bars show SEM, n=5.

References

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