## *In vivo* evaluation of an inducible MRI reporter gene

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Synopsis

Here we report the successful introduction of a novel, *in vivo* inducible MRI reporter gene. *E. coli* was used to overexpress different ferritin-like proteins and effects on T2 were monitored. Among the different iron storage proteins, bacterioferritin (Bfr) showed the highest changes. Induction of *bfr* expression in *E. coli* colonized tumors by exogenous injection of an inducer compound resulted in specific signal reduction in pictures of T2 weighted spin echo sequences.

Introduction

Recently the probiotic *E. coli* Nissle 1917 strain was shown to selectively colonize and replicate within solid tumors of mice, resulting in about $10^9$ colony forming units per gram tumor tissue. Using luciferase expression those bacteria could be monitored in living tumor bearing mice and an arabinose inducible promoter system was successfully tested for its applicability *in vivo*. Since detection of light emission is limited by the composition and thickness of the surrounding tissue, this system is limited in its applications as diagnostic marker, we wanted to test whether we can use ferritin-like proteins as reporter genes for MRI.

Results

The ferritin-like protein encoding genes *ftn* (*E. coli*), *bfr* (*E. coli*), and *dps* (*L. monocytogenes*) were overexpressed in iron containing broth cultures of *E. coli* Nissle 1917. Iron uptake and effects on T2 were analyzed (Fig. 1AB) and compared to control strains and among each other.

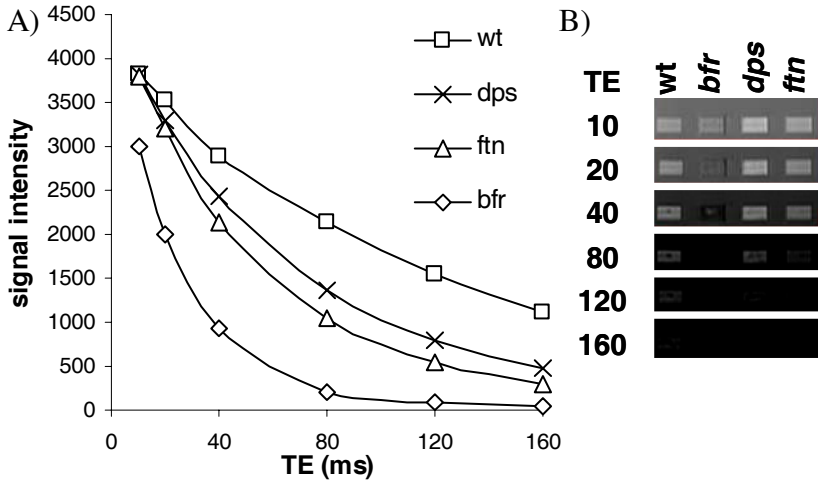


**Fig 1.** A) T2 map of wild-type (wt), *dps*-, *ftn*-, or *bfr*-expressing *E. coli* Nissle 1917 in agarose gels. B) T2 weighted magnetic resonance images.

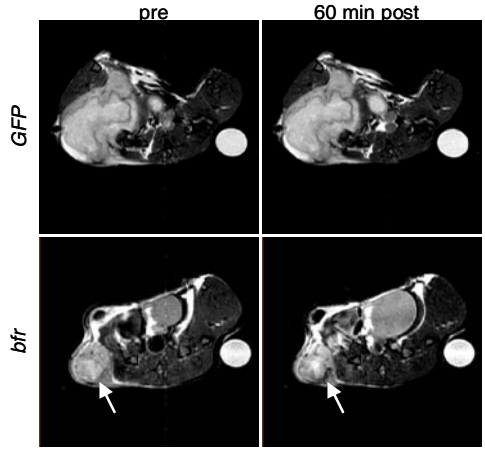


**Fig. 2**. *In vivo* MRI results of reporter gene expression in *E. coli* Nissle 1917 colonized tumors of mice 60 min after induction of gene expression.

The overexpression of all ferritin-like proteins resulted in elevated iron uptake and significant effects on T2, with *bfr* having the strongest effect on both iron uptake and storage as well as effect on T2.

Four days after systemic injection of *E. coli* Nissle 1917 into tumor bearing mice, L-arabinose was administered to induce reporter gene expression. Sixty minutes later significant changes were observed in those mice that were injected with *bfr*-expressing bacteria, while a control strain encoding a *GFP* cassette did not result in any detectable changes when imaging was performed with T2 weighted spin echo sequences (Fig. 2).

Discussion

In contrast to previous publications using ferritin-like proteins as reporter for MRI, we were able to induce and monitor the reporter gene expression in live animals without pre-incubation and saturation in iron-containing media. This probably is based in the different structure of bacterioferritin compared tot the previously used human H-chain ferritin.

Similar to GFP in optical imaging, *bfr* has the potential to become a widely used reporter gene for MRI. When confirmed in other systems it could be used for tracking cells, microorganisms or monitor gene expression in live animals and might also have clinical applications in diagnosis and monitoring of therapeutic effects.