Genetic manipulation of ferritin for enhanced MRI contrast

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Introduction: Superparamagnetic iron oxide agents (SPIO) have been widely used to label and track cells *in vivo,* both in animals models and in the clinical setting (1). However, dilution of the MRI contrast agent as cells multiply, limits the applicability of this method for long-term monitoring of dividing cell populations, and alternative approaches are required for the longer term tracking of haematopoietic cells (2). In this study we present a novel gene delivery strategy that results in the durable production of endogenous contrast in rapidly dividing cell populations.

Genove et al. previously reported enhanced contrast from intra-cerebral injection of adenoviral vectors encoding heavy ferritin (FH) and light ferritin (FL) genes (3). We have adopted a similar approach using lentiviral delivery systems, comparing FH, FL and an enhanced mutant of the ferritin light chain (FLmut). These vectors are designed for durable gene expression in haematopoietic cell lineages and would be ideal for tumour modelling studies or tracking of cellular therapies *in vivo*.

Methods:

Lentiviral vectors (LV): Self inactivating, HIV-1 derived lentiviral vectors encoding the light and heavy ferritin (FL and FH), the mutated light ferritin (FLmut) linked to enhanced green fluorescent protein (eGFP) were generated. Vectors included promoter elements derived from the Spleen Focus Forming Virus (SFFV) long terminal repeat & a central polypurine tract (cPPT) element to enhance transduction efficiency (4). Stocks were prepared by transient transfection with helper plasmids and pseudotyped with the vesicular stomatitis virus envelope. Vector titres were calculated following flow cytometric quantification of eGFP expression following dilutional exposure on a fixed number of target cells.

***In vitro* MRI:** Transduced 293T (>95% eGFP+) cells were grown in iron supplemented media (Ferric citrate 1mM) for 5 days, and washed to remove any excess iron. An aliquot of cells was fixed in 4% formaldehyde and incubated in 2% w/v KCN and 1.35% v/v 12M HCl for 30 mins for Prussian blue staining of iron uptake. The remaining cells were pelleted and scanned in a 9.4T horizontal bore Varian system (72mm volume coil, 256 x 128 matrix, 1mm slice with 2 averages, FOV 60x30mm) using a spin-echo sequence (TR=1.5s, 13 TEs 6-80ms) and a spoiled gradient echo sequence (TR=100ms, FA=30, 11 TEs 2-26ms). R2 and R2* maps and values were derived from a ROI at the centre of each cell pellet using the ImageJ MRI Analysis Calculator plugin and the reciprocal math command.

Results: Gene transduction was confirmed by flow cytometry for eGFP and MRI reporter gene expression was evaluated by western blot analysis (data not shown). Cells expressing FH and FLmut, but not FL were readily detectable in T2-weighted images and R2 maps (Figure 1). Figure 2 displays average R2 values of these samples. Figure 3 demonstrates positive Prussian blue iron staining inside cellular bodies of transduced cells (arrows).

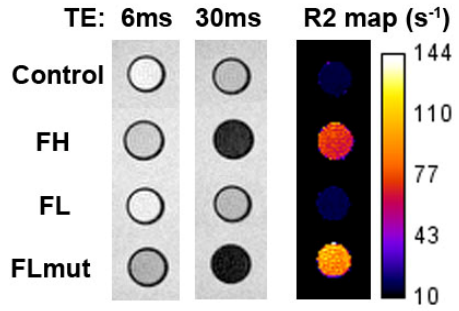

Figure 1. T2-weighted images and R2 map

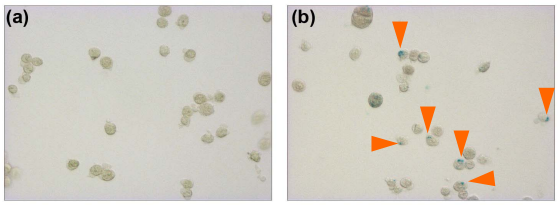

Figure 3. Prussian blue iron stain
a) non-transduced cells b) transduced cells

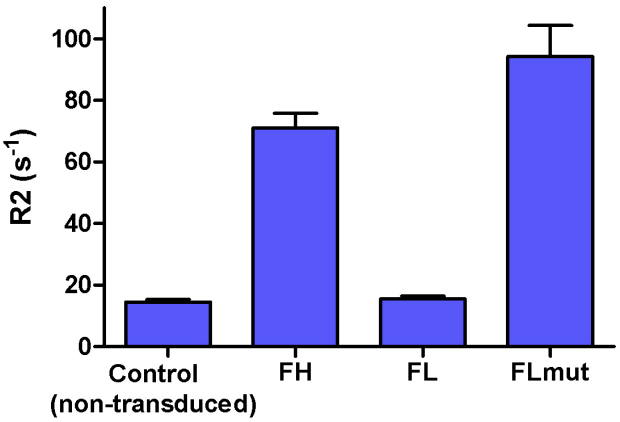

Figure 2. R2 values

Discussion: The ability to durably label and track dividing cells *in vivo* is attractive for studies of tumour modelling, and cell migration and homing. We report that lentiviral mediated manipulation of ferritin expression can produce marked image contrast within dividing cells, and describe a codon optimised variant of the human light ferritin gene (FLmut) which further increases MRI conspicuity.

References:

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