Gene based production of magnetic nanoparticles for MRI

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Introduction:

Molecular imaging provides information on the cellular and molecular level and holds great potential for more specific and timely detection of disease. For optical imaging, fluorescent methods have long been used for these purposes and gene-based methods such as the expression of green fluorescent protein (GFP) have been utilized as genetic markers for expression and tracking studies. For MRI, normal iron homeostasis proteins have been explored, and ferritin and its derivatives have been shown to be potential markers for MRI. Here we describe an approach to molecular and cellular imaging with MRI utilizing *in vivo* magnetic nanoparticle production within cells expressing the gene for MagA, an iron transporter in naturally occurring magnetotactic bacteria. This approach extends the use of MRI as a tool to study *in vivo* cell biology and for monitoring therapies based on the use of stem cells and progenitors.

Methods:

The *magA* gene was cloned into a lentiviral vector under the control of the minimal immediate early promoter of cytomegalovirus ($_{mini}CMV$) which can then be induced via doxycycline. Replication defective lentivirus was generated and used to infect 293FT cells. After several cell passages to ensure stable integration, single cells were picked and expanded to create clonal cell lines. PCR was performed to confirm the presence of the *magA* gene to identify positive clones. For transmission electron microscopy (TEM), *magA* cells were cultured with 2 μg/mL doxycycline and 200 μM Fe for four days. The cells were then fixed, dehydrated and embedded in Eponate resin. Ultrathin sections (60-70 nm) of cells were observed on a Hitachi H-7500 transmission electron microscope without counterstaining. *In vitro* relaxivity measurements of cell pellets were made on a 3T Siemens TRIO.

For *in vivo* experiments, uninduced 2B5R cells were transplanted into the mouse striatum shown on the right in the images below. The contralateral side was transplanted with a 293FT cell line which did not express *magA*. In each case, the transplantation was made using a stereotaxic platform fitted with an automated injector and Hamilton syringe and using 10^5 cells suspended in a total volume of 4 μl in phosphate buffered saline (PBS). Three mice were studied as described below. For all three mice, cells were allowed to grow for five days from the day of surgery, during which doxycycline was administered in the drinking water at 5 mg/mL and in the food at 200 ppm. On day 5, one mouse was imaged and sacrificed according to IACUC animal care protocol. The remaining two mice were kept for an additional eight days without administration of doxycycline. On day 13, the second mouse was imaged and sacrificed. The third mouse was maintained for an additional 6 days, during which doxycycline was administrated again in food and drinking water. On day 19, this mouse was imaged and sacrificed. In all cases, imaging was performed under anesthesia (1.5% isofluorane) on a 9.4T Bruker MR system fitted with a heated mouse cradle. MRI parameters: FLASH sequence, TE 6.0 ms, TR 50 ms, FOV 256×256, 88 μm in-plane, slice thickness 0.450 mm.

Results and Discussion:

Relaxation measurements made on cell pellets revealed that cells expressing *magA* and supplemented with iron exhibit significantly increased R2 (17.5 ± 1.0 vs. 4.3 ± 0.1 s^{-1} for control cells). Figure 1 shows TEM images of these cells, revealing particles of approximately 3-5 nm in diameter and spherical in shape. They are most easily identified within endosome-like organelles. The multivesicular nature of these organelles suggests they may eventually go through the cellular degradation pathway. These particles have been isolated and found (by X-ray diffraction) to consist mainly of iron oxide in the form of magnetite. Figure 2 shows the results of the *in vivo* imaging experiment for 3 mice. The green arrows (control cells) and red arrows (*magA* expressing cells) indicate the location of the burr hole for cell transplantation. Panel (a) shows that *magA* expressing cells can be easily identified by MRI on the left side of the image. In panel (b), a second mouse, which has been maintained without gene induction for eight days, following the initial five days of induction, is shown. Here the transplanted cells cannot be seen by MRI. This suggests the particles formed during the original induction may have been degraded. Panel (c) shows that by reintroducing doxycycline, transplanted cells are again found by MRI. This suggests that the cells are still present and capable of reforming magnetic nanoparticles. Taken together, these results show that cells expressing *magA* can be tracked by MRI, that by turning the gene "off", the contrast can be diminished, and that by again turning the gene "on", cells can again produce MRI contrast. These results suggest *magA* can be used as a long-term MRI gene marker.

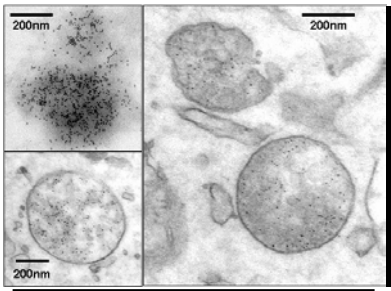

Figure 1: TEM images of *magA* expressing cells.

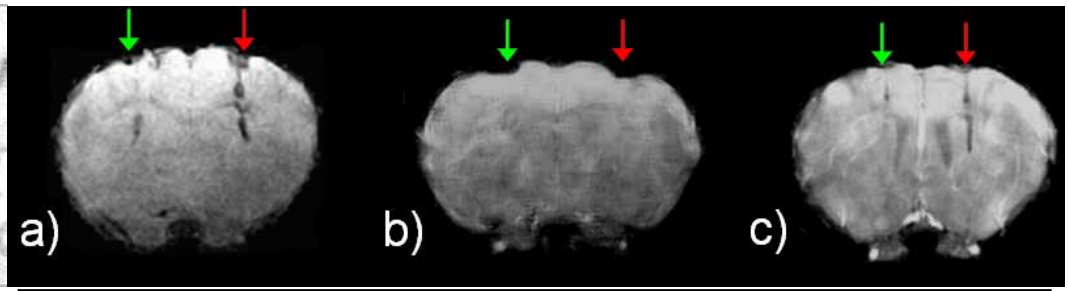

Figure 2: a) mouse imaged on day 5 post-transplantation, **b)** mouse imaged on day 13 (5 days gene induction, followed by eight days without), **c)** mouse imaged on day 19 (5 days induction, followed by 8 days without, followed by 6 days induction).

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