Repetitive Imaging of Tumor Cell Growth Using Gene-based, Iron Contrast: MagA vs. Modified Ferritin Subunits

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Introduction Tracking endogenous cell contrast by MRI remains a challenge for molecular imaging, where structural detail depends on efficiency of contrast gene expression, hardware limitations and scan time. We examined repetitive imaging of human breast/melanoma cancer cell growth in mouse xenografts, comparing iron contrast from MagA expression (1) with that of the modified ferritin subunits (HF+LF, 2), lacking iron response elements, and parental controls. This study exposes differences in MR contrast formed by these iron binding proteins and their influence on tumor cell tracking.

Methods Clonal cell lines were obtained from genetically engineered MDA-MB-435 cells using standard techniques. All cells were cultured in media supplemented with 250 μM ferric nitrate for 2 days prior to subcutaneous injection into the hind limbs of nude mice. Ten million cells in Matrigel were introduced at each injection site and monitored by repetitive MRI, optimized for two phases of cancer growth: (I) nonpalpable tumors, < 2 weeks post-injection; and (II) palpable tumors, 2-5 weeks post-injection. Animals were anesthetized with isofluorane and imaged 3 days post-injection and at weekly intervals. Tumor samples were collected and flash frozen after each imaging session. For each expression system, n=8 mice.

Results Images were collected and analyzed in 3 ways: a) CNR comparing the ratio of signal (void) to noise throughout the tumor, b) fractional signal loss comparing signal intensity/voxel of parental and engineered tumors, and c) fraction of tumor volume attributed to signal void. Figure 1 shows phase I growth from HF+LF and parental cells. Within a few days, irregular tumor morphology is evident in aggressively growing cancer cells. Tumor contrast from engineered cells shows discrete regions of signal void not seen in parental tumors.

Discussion Our results provide MR imaging parameters for tracking endogenous cellular contrast using custom, high performance RF coil and gradient insert on a 3T clinical scanner. A two-phase model of cancer growth helps maximize MRI resolution over a local region of interest, thus providing greater structural detail for cellular and molecular imaging of micro-tumors. In addition to cancer stem cell biology, these data are relevant to cell transplantation research and optimization of reporter gene expression for live animal MRI and PET/MRI.

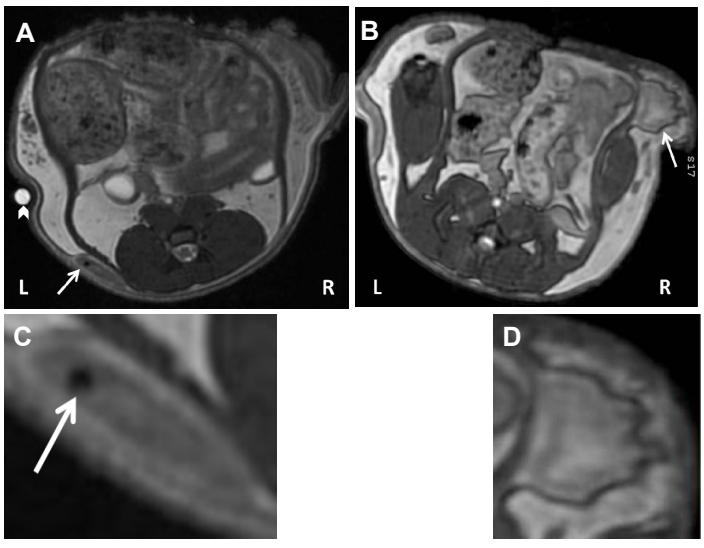

Figure 1. MRI detection of tumors 3 days post-injection. Axial cross sections of anesthetized mice show early stages of tumor growth from cells expressing HF+LF (**A**) or parental controls (**B**). Mice are oriented with their back down and head toward the viewer. A water tube (arrowhead) distinguishes left (L) from right (R). Arrows in **A** and **B** point to tumors (**C** and **D**, respectively) that were barely palpable (volumes approximately 25 mm^3 by caliper). Several regions of signal loss were detected in the HF+LF expressing tumor (arrow in **C**). Using a 3D balanced-SSFP pulse sequence, mice were scanned at 3T with a custom built RF coil and gradient insert (3). SNR of muscle was 52 (**A)** and 64 (**B)**. Scanning parameters were as follows: FOV=3 cm, slice thickness=200 μm, BW=±62.5, flip angle=20, TR/TE=3.8/1.9 ms (**A**), TR/TE=3.2/1.6 ms (**B**) and 2 averages. At higher in-plane resolution (150x150 $μm^2$, **A**) SNR comparable to lower resolution, isotropic images (200x200x200 $μm^3$, **B**) was obtained in a reasonable scan time by increasing the RF phase cycling from10 (**B**) to 12 (**A**).

References

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