Quantitative MRI reporter gene imaging of the recruitment of ferritin over-expressing fibroblasts to the vascular niche of solid tumors

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Introduction We used the over-expression of human ferritin heavy chain (FHC) as an MRI reporter gene, R2 mapping with bi-exponential analysis, and measurement of fractional blood volume (fBV) using an intravascular gadolinium (BSA-Gd) contrast agent for quantitative *in vivo* tracking of fibroblast recruitment to the vascular niche within solid ovarian carcinoma tumors. **Methods** Tumors were generated via subcutaneous injection of $4x10^6$ human ovarian carcinoma (MLS) cells into the hind limb of CD1-nude female mice. In order to examine 2 models of fibroblast recruitment, either (i) MLS cells were co-injected (CoI) with 10^6 CV1 fibroblasts (Control, n = 6), 10^6 FHC over-expressing CV1 (CV1-FHC) cells (Ferritin, n = 7), or 10^6 CV1-FHC cells exposed to 1mM ferric citrate (FC) for 48 hours prior to implantation (Ferritin+FC, n = 6), or (ii) $2x10^6$ CV1 (Control, n = 7) or $2x10^6$ CV1-FHC (Ferritin+FC, n = 7) were delivered remotely via intraperitoneal (IP) injection 4 days after tumor initiation (Recruitment). Multi-slice multi-spin echo (MSME) MRI (TE=7.7ms*30echos, TR=3s, FOV= 2.8x2.8cm, Matrix=256x256, AV=3, slices=5-8) was performed on a 9.4T scanner (Bruker, Germany) at multiple time points (Fig A). R2 was quantified via fitting signal waveforms to $I=I_0e^{-R2*TE}+C$. The R2 of CV1-FHC cells ($R2_{fhc}$) was quantified using CV1-FHC cell phantoms. Bi-exponential analysis was performed on a pixel-wise basis using the equation $I=I_0(Ae^{-TE*R2fhc}+Be^{-TE*R2c})+C$, where $R2_c$ is the mean R2 of all control tumors. The CV1-FHC fraction was calculated as A/(A+B)*100. In CoI studies, fBV was quantified following intravenous infusion of BSA-Gad (n=3 per group) as described in [1]. Immuno-histochemical and immuno-fluorescent staining were performed at the conclusion of each study. **Results** R2 mapping revealed preferential recruitment of CV1-FHC cells to the tumor rim in both models (Fig A,B). Bi-exponential relaxometry (Fig B) revealed CV1-FHC cell fractions of 39.5±5.9% (CoI) and 44.8±5.5% (Recruitent) at the rims of Ferritin+FC tumors, in agreement with quantitative immuno-fluorescent measures of 49±7% and 46±7% in identical tumors. Vascular mapping revealed an fBV dependent distribution of R2 values in Ferritin and Ferritin+FC tumors (Fig C), with an increased frequency of high R2 values (R2>28 (1/s), red dotted line) at increasing fBV values. **Conclusions** FHC over-expression enabled *in vivo* imaging of fibroblast recruitment to solid tumors (Fig A). When combined with bi-exponential analysis, the CV1-FHC cell fraction could be measured within a mixed cell population (Fig B), representing a significant improvement over conventional fluorescence or bioluminescence reporter gene methods that report only on the total of labeled cells but not their relative fraction. The correlation of fBV and R2 in Ferritin and Ferritin+FC tumors (Fig C) revealed the preferential recruitment of CV1-FHC fibroblasts to the peri-vascular niche within growing tumors.

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[1] Dafni et al. NMR Biomed. 2002;15(2):120-31.

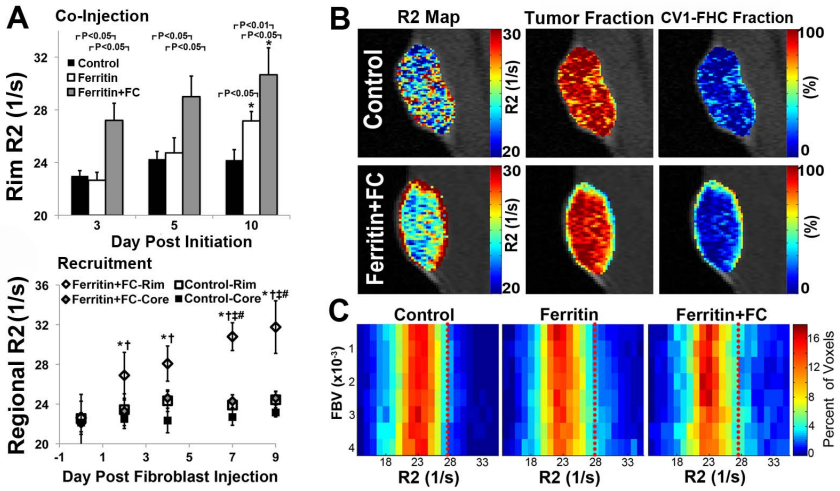

Figure. (A) Regional R2 measurements in CoI (top) and Recruitment studies (bottom) revealed preferential recruitment of CV1-FHC fibroblasts to the rim of tumors (*P<0.05 vs. Core, †P<0.05 vs. Control, ‡P<0.05 vs. Day 0 post injection, #P<0.05 vs. Day 2 post injection). (B) Representative R2 and cell fraction maps generated using bi-exponential fitting of tumor relaxation data reveal high CV1-FHC cell fraction along the rim of the Ferritin+FC tumor. (C) Heat maps of R2 distribution as a function of fBV reveal an fBV dependent increase in high R2 values (red line) in Ferritin and Ferritin+FC tumors, but not control tumors.