MR Imaging of Tumor Initiating Melanoma Cells

S. Magnitsky[1], A. Roesch[2], S. Pickup[1], M. Herlyn[2], and J. D. Glickson[1]
[1]Radiology, University of Pennsylvanian, Philadelphia, PA, United States, [2]Wistar Institute, Philadelphia, PA, United States

Introduction: *In vivo* and *in vitro* investigations of malignant melanoma revealed substantial tumor heterogeneity [1]. Several sub-populations of melanoma cells with high tumorogenicity, self-renewal capacity and resistance to treatments have been identified [2]. Proliferation studies of these "tumor-initiating" cells indicate that they a slowly-cycling phenotype. The chromatin remodeling factor JARID1B was identified as a new molecular marker of slowly-cycling melanoma cells. The goal of this study was to develop a technique to identify and track these tumor initiation cells *in vivo*. We utilized the effect of slowly proliferating cells to hold high concentration of contrast agent. After initial uptake of the contrast agent, cells with normal cycle dilute out iron due to division, which leads to essentially total disappearance of the label. Tumor initiating cells divide at a much slower rate than bulk melanoma cells. Undivided cells preserve a high concentration of iron oxide particles, which can be detected by *in vivo* MR imaging.

Methods: Human melanoma cells (WM3734, WM3918, WM35, 1205Lu) were labeled with iron oxide particles of different sizes (USPIO (30 nm), SPIO (150nm), MPIO (860 nm)). Toxicity of iron oxide particles on melanoma cells was tested in MTT assays and colony formation experiments. The presence of slow proliferating cells was confirmed microscopically and by flow cytomentry. Drug resistance and tumorigenicity of slow proliferating cells were confirmed *in vitro*. In order to show the feasibility of *in vivo* detection of tumor initiating cells, WM3734 melanoma cells were labeled with 100 µg/ml of iron oxide particles *in vitro* and 1 million of labeled cells was xenografted into NOD/SCID mice. Tumors were grown for 8 weeks and MR imaging was performed at 4.7 T magnet. Imaging parameters: 2D gradient echo with TR/TE=200/5 ms, resolution 156x156x1000 µm. After *in vivo* experiments animals were sacrificed and excised tumor tissues were imaged at 9.4 T magnet. Imaging parameters: 3D gradient echo with TR/TE = 200/5 ms, resolution 80 µm isotropic. The presence of iron-retaining cells was confirmed by Prussian blue staining. The iron concentration per cell was measured by inductively coupled plasma mass spectroscopy.

Results: Overnight incubation of melanoma cells with of iron oxide particles leads to ~100% of cells labeling, with an average iron concentration of 87, 42, 16 pg of Fe/cell for MPIO, SPIO and USPIO particles respectively. Iron labeled cells exhibit the same proliferation and tumorigenicity as unlabeled melanoma cells. To test the hypothesis that slow cycling cells retain the "original" concentration of contrast agent, we allowed labeled cells to proliferate for several weeks. The number of iron-retaining cells was estimated by flow cytometry. A distinct (1-2%) iron-retaining sub population was detected after 20 days of proliferation (Figure 1). Semi-quantitative RT-PCR test of iron-retaining cells revealed the highest expression of JARID1B protein in the most iron-positive cells, while iron-free cells expressed lower amounts of JARID1B ($p<0.05$). In order to demonstrate that iron-retaining cells can be imaged, we labeled WM3734 melanoma cells with iron oxide particles. Two separate subcutaneous injections of iron-labeled and unlabeled (control) cells were performed in NOD/SCID mice. Xenografts were grown for ~ 8 weeks and animals were MR imaged in a 4.7 T magnet. *In vivo* MR images of tumor xenografts clearly showed regions of hypointensity, while control tumors (grown from unlabeled cells) did not show a similar pattern (Figure 2-I). *Ex vivo* MR images of tumor tissue grown from iron labeled cells exhibited a different contrast compare to control tumors: more hypointense regions were detected in the iron positive tumor, and the presence of iron-oxide particles was confirmed by Prussian blue staining (Figure2-II, III).

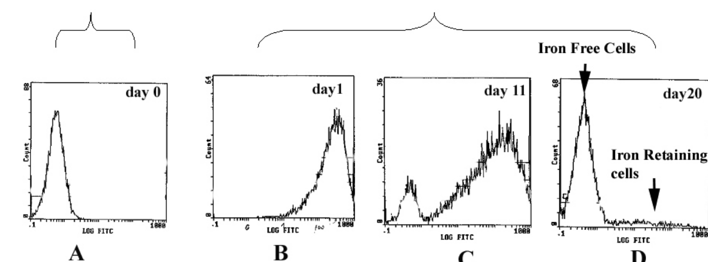

***Figure 1. Detection of iron-retaining melanoma cells by flow cytometry**. Unlabeled melanoma WM3734 cells (A) were incubated with iron oxide particles at day 0 for 12 hrs. 100% iron-positive cells was detected at day 1 after labeling (B). Dilution of iron oxide particles due to cell division and formation of two distinct populations, iron positive and iron free, was observed at day 11 after labeling (C). A small distinct population of iron-retaining cells was detected after 20 days of proliferation (D). Panel D. Left peak shows cells with undetectable amount of iron oxide particles (see to panel A). Right peak shows cells with undiluted concentration of contrast agent.*

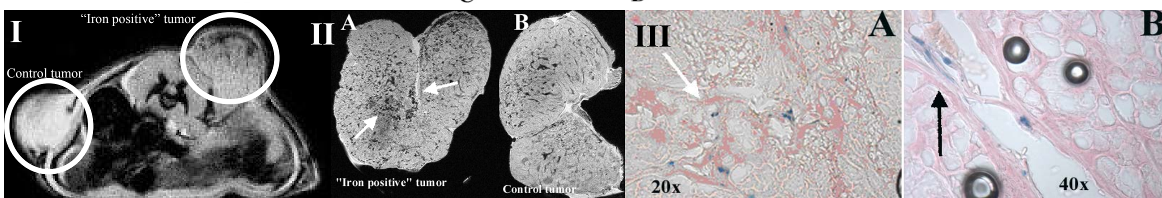

***Figure 2. (I) In vivo MR image of tumor initiating cells.** Gradient echo MR image of mouse with tumor xenografts was taken 2 months after implantation of one million of SPIO labeled MW3734 cells.*
***(II) Ex vivo detection of tumor initiating cells**. Ex vivo MR images of tumor tissue. (A)Tumor grown from iron labeled cells. (B) Tumor grown from control/unlabeled cells. Tumor vasculature is clearly seen on both MR images. Arrows on panel A pointing on "punctured" spots due to iron-retaining cells. **(III) Histological detection of iron-labeled cells in tumor tissue.** Prussian blue staining of tumor tissue grown from iron-labeled cells. Iron-positive cells (blue) were detected in tumor tissue. No iron-positive cells were detected in tumors grown from unlabeled cells (not shown). (A) 20x (B) 40x magnification.*

Discussion and Conclusion: Dilution of iron oxide particles following cell proliferation has been reported [3, 4]. We used this property of MR contrast agents to identify melanoma slowly-cycling cells. Uniform labeling with MRI detectable iron can be achieved after 12 hours of melanoma cells incubation with iron oxide particles. The presence of an iron-retaining sub-population was detected after 20 days of the cells proliferation *in vitro* and after 8 weeks of tumor growth *in vivo*. This slow proliferating sub-population is drug resistant, highly tumorogenic and showed increased expression of JARID1B which is a new biological marker of melanoma cells with enhanced tumor self-renewal potential (Roesch A et al., *submitted*). The iron-retaining cells were detected by *in vivo* and *ex vivo* MR imaging. These findings provide a simple practical method for investigating properties of tumor initiating cells *in vivo*, and open an opportunity to test the cancer stem cells hypothesis.

Reference: 1.Rastetter, M., et al., Histol Histopathol, 2007. **22**(9): p. 1005-15.**2**.Zabierowski, S.E. et al.,J Clin Oncol, 2008. **26**(17): p. 2890-4. **3**.Walczak, P., et al., MRM, 2007. **58**: p. 261-69.**4**.Magnitsky, S., et al., Neuroimage, 2005. **26**(3): p. 744-54.