Iron Retention in Nonproliferative Cancer Cells Allows for Tracking by MRI: An In Vivo Assay for Studying Cancer Cell Dormancy

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INTRODUCTION: Cell tracking with MRI and iron nanoparticles is commonly used to monitor the fate of implanted cells in disease models. Few studies have employed these methods to study cancer because proliferating cancer cells will lose the iron label as they divide. In this research, we demonstrate the ability to use retention of iron nanoparticles by non-proliferative cancer cells, and the resulting MRI signal void, to serve as a marker for cancer cell dormancy. Dormancy is a stage in cancer progression where cancer cells exist in a viable but quiescent state. Dormant cancer cells may be the most dangerous of all since they can be triggered to proliferate some time later, may contribute to cancer recurrence, and are not affected by therapy targeting proliferating cells. Our lab has developed technology to track single iron-labeled cells over time [Heyn *et al* (2006) Magn Reson Med]. Here we present 3 studies where this cell tracking technology was applied to investigate the fate of cancer cells *in vivo*. We also present experiments designed to validate that discrete, persistent signal voids in our images represent non-proliferative cancer cells.

STUDY 1: CHARACTERIZATION OF NONPROLIFERATIVE HER2+ BRAIN METASTATIC BREAST CANCER CELLS IN MICE. Six female nude mice were given an intra-cardiac (IC) injection of 175,000 iron-labeled, human brain-seeking breast cancer cells (MDA-MB-231-BR-HER2) into the left ventricle of the heart. MRI was acquired at 3T using 3D bSSFP (resolution: 100x100x200 µm, scan time = 32 min) on day 0, 4, and 32 to evaluate signal voids due to iron loaded cells and development of metastases. The number of signal voids was measured in 22 image slices evenly distributed throughout the brain. Fractional signal loss (FSL) (mean SI of background tissue – mean SI of void / mean SI of background) was measured in a subset of voids that persisted from day 0 to 32. We have previously shown that the FSL, a measure of the contrast due to iron-labeled cells, is related to the iron/voxel and can be used to determine if the amount of iron in voids changes over time. *Results:* Most of the signal voids were cleared from the brain over time. The percentage of voids persisting in the brain relative to day 0 was on average 33% on day 4 and 16% on day 32 (**Figure A**). These voids are attributed to cancer cells that have not divided and so retain the iron label over time. The average FSL of persisting voids did not change significantly between day 0 and day 32, suggesting that these cells are not proliferating (**Figure B**) despite other cells forming tumors.

STUDY 2: INVESTIGATION OF RADIOTHERAPY ON NONPROLIFERATIVE HER2+ BRAIN METASTATIC BREAST CANCER CELLS IN MICE. Cancer cells were administered to six female nude mice and scanned using MRI for analysis of signal void and FSL as in study 1. All mice were treated with whole brain radiotherapy (WBRT) (20/2 Gy) on days 1 and 2 post cell injection. *Results:* Representative images of mouse brain over time are shown in **Figure C**; voids are indicated with black arrows and tumors in yellow. The percent of voids remaining relative to day 0 was 34% at day 4 and 11% at day 32. The number of signal voids was not significantly different between treated and untreated mice at any time point (**Figure D**), although tumor burden – both in terms of number and volume - was significantly different between the two groups (**Figure E, F**). The FSL did not change significantly between day 0 and 32 nor was it different compared to untreated at either time point; an example is shown for WBRT-treated mice (**Figure G**) This suggests that non-proliferative cancer cells persist in the brain, seemingly unaffected, even after WBRT has eradicated the tumor burden. This is not surprising as radiation first kills actively dividing cells. Cells that are in 'resting phase' or are slowly dividing are far less radiosensitive. This further supports that we are tracking nonproliferative cells.

STUDY 3: CHARACTERIZATION OF GLIOMA CELLS WITH DIFFERENT DORMANCY PROFILES. Three novel glioma cell lines were investigated. After direct injection into the brain U373vIII cells rapidly grow tumors (30-50d) and have no dormant phase; TF (tissue factor) U373 cells have a long latency period with a dormant phase of 70+ days; EV (empty vector) U373 cells remain dormant for more than 250 days and do not grow tumors. We used these 3 cell lines in our model system where cells (200,000) are iron-labeled and then injected IC for delivery to brain. Images were acquired as above on day 0, 15, 30 and 50 or 70 to monitor signal voids and tumor development. *Results:* For U373vIII mice, the number of signal voids declined slowly and persisted through day 50 when tumors became evident in 3/6 mice. For TF U373 mice, the number of signal voids declined rapidly between day 0 and 15 and by day 30 no signal voids were detectable; these mice were scanned again on day 70 and neither voids nor tumors were evident. The pattern of void retention was similar in EV U373 mice and these also did not grow tumors by day 70. This suggests that TF and EV U373 cells do not grow brain tumors in this model because cells do not survive and are rapidly cleared; it is not because they remain in the brain as dormant cells.

VALIDATION STUDIES: (A) VOIDS CORRESPOND TO IRON-LABELED CANCER CELLS. Brains removed 21 days after IC injection of cancer cells and stained with Perls Prussian Blue to detect iron revealed the presence of iron-positive cells persisting in the brain. This indicates that MRI signal voids are the result of iron-labeled cells (**Figure H**). **(B) VOIDS REPRESENT NONPROLIFERATIVE CANCER CELLS.** Mice received IC injections of either viable iron-labeled cancer cells or cancer cells rendered apoptotic by doxorubicin. MRI was acquired on day 0 and 14. Dead cells did not persist in the brain, they were mostly cleared by day 14 (**Figure I,** note: a threshold has been applied to this image to better visualize voids), suggesting that the voids we are tracking are live, nonproliferative cells.

SIGNIFICANCE: Dormant cancer cells are believed to provide a reservoir of viable cancer cells that evade treatment and contribute to tumor recurrence. At present, there are no unique markers to identify or monitor these cells. As a result, critical questions about what triggers a dormant cell to proliferate into a clinically relevant tumor have remained unanswered. Our technique to track and monitor the fate of individual non-proliferative cancer cells *in vivo* provides a unique way to investigate this poorly understood cancer cell population. This research is important to improve our understanding of cancer dormancy and prevent tumor recurrence in patients.