The Use of Cellular MRI and Magnetic Particles to Study Cancer Stem Cells

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Background: The most deadly aspect of cancer is its ability to spread, or metastasize. 10-20% of metastatic breast cancer patients will develop brain metastases A diagnosis of brain metastases is terrifying. Untreated, the median survival time is 2–3 months and with aggressive treatment is extended only marginally, to 4–12 months. An important, but as yet unexplored, area is the role of stem-like cancer cells in determining the metastatic phenotype. The so-called cancer stem-cell (CSC) is thought to possess the ability to produce progeny of both stem-cell and differentiating fates, resulting in tumors of a heterogeneous phenotype. Recent evidence suggests that stem cells are the culprits of metastasis, supported by the observation that metastatic tumors tend to reproduce a similar heterogeneity as the primary tumor. Here we describe the technology developed in our labs for tracking CSC, in a mouse model of breast cancer metastasis to the brain, using MRI and magnetic particles.

Methods: A human breast cancer cell line previously selected for brain tropism and transfected with an EGFP construct (MDA-MB-231BR) was sorted by flow cytometry using fluorescent antibodies directed against CD44 and CD24. 24h after the sorting, the cells were incubated with either micron sized iron oxide particles (MPIO, Bangs beads, 0.9µm, Flash red) or superparamagnetic iron oxide particles (SPIO, MoldayION rhodamine B, 50nm) at 2 different concentrations (50 and 100µg/mL) for 24 hours. Unsorted cells were prepared and labeled at the same time. Cytotoxicity was evaluated using the trypan blue exclusion assay and by examining plating efficiency after 9 days. Fluorescence imaging and Perl's Prussian blue staining for iron were used to visualize iron within cells. 175 000 Molday-labeled cells were injected in the left ventricle of the heart in nude mice. MRI was performed 24 hours after their injection using a steady state free precession imaging sequence (SSFP) (TE/TR=13/26ms, flip angle=35°, rBW=±11kHz, FOV=1.5cm, matrix=150x150, thickness=0.2mm, NEX=2, acquisition time=41min) on a clinical 1.5 Tesla scanner equipped with a custom-built high performance gradient coil.

Results: The 231BR human breast cancer cell line can be sorted by flow cytometry into two distinct populations: CD44high/CD24low and CD44low/CD24high, representing the CSC-like and non-CSC cells respectively [1]. Figure 1 shows the CD24/CD44 expression in 231BR cells along with the nonspecific IgG isotype control. Cells could be labeled with both MPIO and Molday. However, the sorted cell populations had lower viability and lower plating efficiency after labeling with the larger Bangs beads (Figure 2). Viability after labeling with either concentration of Molday was very similar to that of unlabeled cells. With Molday labeling, the cells can be visualized by both MRI and by fluorescence (Figure 3). After Molday labeling, very little free iron remains in the extracellular space, unlike MPIO. Molday labeled CSC can be detected *in vivo* in SSFP images of the mouse brain after intracardiac injection (Figure 4).

Fig 1. Flow cytometry showing the CD24/CD44 expression in 231BR cells

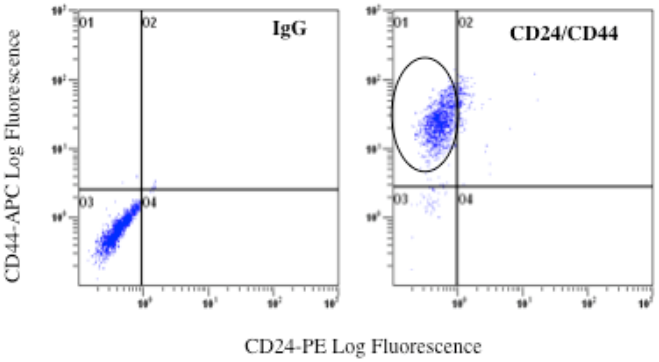


Fig 2. Average viability of cells after labeling +/- StDev

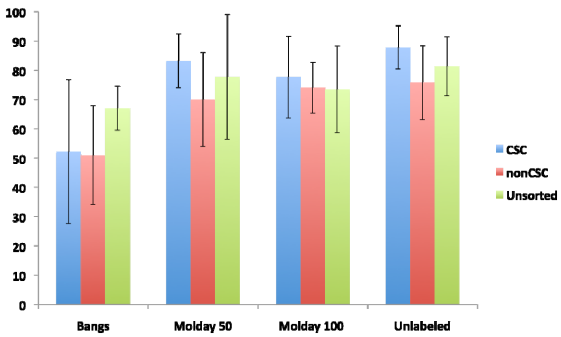


Fig 3. PPB and Fluorescence of Molday labeled CSC

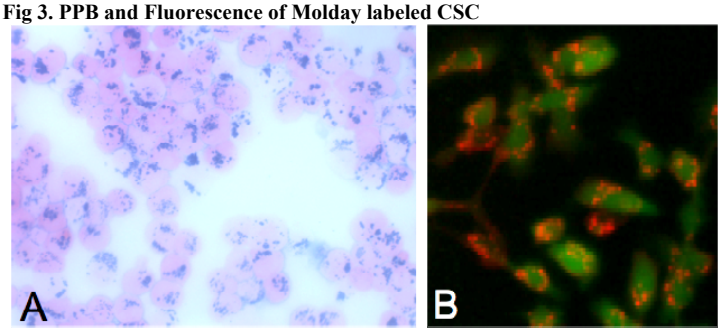
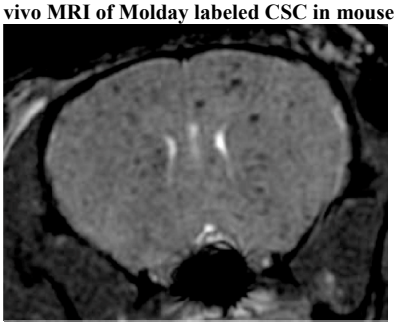


Fig 4. In vivo MRI of Molday labeled CSC in mouse brain



Summary: With evidence emerging in support of a cancer stem-cell model of carcinogenesis, it is of vital importance to identify and image these elusive cells. The cancer stem-cell hypothesis has the potential to explain unresolved questions related to tumor initiation, tumor heterogeneity, chemotherapeutic resistance, and even the metastatic phenotype. We have developed tools and models for *in vivo* tracking of stem-like cancer cells. The sorted cancer cell populations can be labeled efficiently and without significant toxicity with the new commercially available iron agent MoldayION Rhodamine B, which also allows validation of image data by fluorescence microscopy. Our model of breast cancer metastasis to the brain involves the injection of cancer cells into the left ventricle of the heart in nude mice for delivery to the brain. The cancer cells can be detected in the brain immediately after the injection, at their initial arrest in the brain as regions of signal void, and their presence, location and the metastases that develop can be tracked over time. The tracking of cells at the single cell level is achieved using a customized micro-imaging system at 1.5 Tesla, which includes the use of a custom-built gradient coil insert and a 3DSSFP imaging sequence. SSFP is very sensitive to the intravoxel dephasing caused by intracellular iron and is also the most SNR efficient of all pulse sequences, allowing very high SNR images at the relatively low field strength. The gradient insert allows fast, high resolution imaging of the mouse brain. This MR technology was developed and optimized previously in our lab to permit the *in vivo* detection of single cells in mouse brain [2]. To the best of our knowledge there are no other labs in the world using MRI to study cancer metastasis or cancer stem cells *in vivo* at the single cell level.

[1] Croker A *et al*, J Cell Mol Med 2008 ; [2] Heyn C *et al*, Magn Res Med 2006