Longitudinal evaluation of MPIO-labeled stem cell biodistribution in a GBM model using MR imaging and DCE-MRI at 14.1Tesla

M. M. Chaumeil[1], C. G. Boyd[2], B. Gini[3], R. Santos[2], J. de la Torre[2], C. Ng[2], H. Yang[3], A. Iwanami[3], S. Sukumar[1], T. Ozawa[2], R. O. Pieper[2], P. Mischel[3], C. D. James[2], and S. M. Ronen[1]

[1]Radiology, University of California, San Francisco, San Francisco, CA, United States, [2]Neurological Surgery, University of California San Francisco, San Francisco, CA, United States, [3]Pathology & Lab. Medicine, University of California Los Angeles, Los Angeles, CA, United States

INTRODUCTION

Despite advances in surgical, radio-, chemo- and gene therapies, the median survival of newly diagnosed glioblastoma (GBM) patients remains under 15 months. The main reason is that these therapies are unable to reach all tumor cells, particularly tumor microsatellites disseminated in normal brain. An interesting property of stem cells (SCs) is their high pathotropism in the brain, including towards brain tumors (1). SCs modified to express therapeutic genes thus present a promising new approach for the treatment of GBMs. In this context, the purpose of this study was to characterize the pathotropism of two different stem cell sources toward GBM tumors, human mesenchymal stem cells (hMSCs) and fetal neural stem cells (fNSCs), in order to determine which cell source provides the best tumor coverage and would consequently be the best candidate for SC-based therapy. To do so, high resolution MR imaging at 14.1T was used to longitudinally monitor micron size paramagnetic iron oxide particle (MPIO)-labeled SC distribution in a GBM tumor-bearing mouse model.

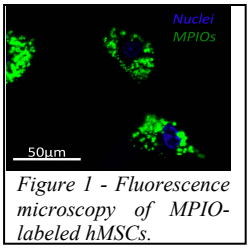

Figure 1 - Fluorescence microscopy of MPIO-labeled hMSCs.

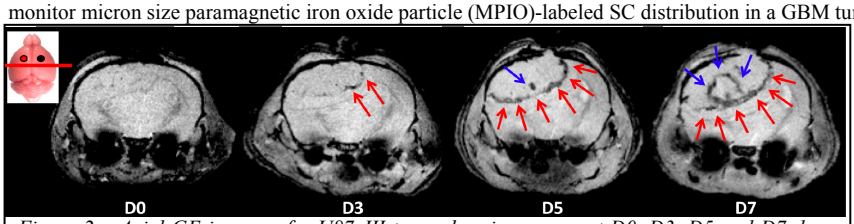

Figure 2 – Axial GE images of a U87vIII tumor bearing mouse at D0, D3, D5 and D7 days post injection of MPIO-labeled hMSCs. The slice is localized 0.8mm posterior to the injection sites of tumor cells (● insert) and stem cells (○ insert). Red arrows show the localization of MPIO-labeled hMSCs at the rim of the tumor, blue arrows the stem cells located inside the tumor mass.

MATERIAL & METHODS

Stem cells labeling hMSCs (San Bio, Inc, CA) and fNSCs were labeled with 1.63µm diameter MPIO (Bangs Laboratories, Inc, IN) as previously described (2,3). Briefly, MPIO suspension was added to the growth medium (10 µL/mL) and cells were incubated overnight. MPIOs not taken up by cells were removed by multiple washes with PBS. Confocal fluorescence microscopy was used to confirm MPIO cytoplasmic localization and lack of free MPIO in media.

Tumor-bearing animals & stem cells injections 5-week-old athymic mice (Nu/Nu, Simonsen Laboratories, Gilroy, CA) were used in this study. For tumor implantation (n=13), animals were anesthetized using ketamine/xylazine (100/20mg.kg^{-1}resp.) and a suspension of U87vIII cells (EGFR overexpressing GBM cell line, ~3x10^5 in 3µl) was injected into the right caudate-putamen of the mouse brain (3). A week post tumor implantation, animals were injected with MPIO-labeled hMSCs (n=5) or fNSCs (n=8) (~1x10^6 in 3µl) either directly inside the tumor or in the contralateral hemisphere following a protocol similar to tumor cell injections.

MR system Experiments were performed on a 600 MHz wide bore vertical system (Ø$_i$=55 mm) equipped with 100 G.cm^{-1} imaging gradients (Varian Inc, Palo Alto, CA). Shimming and MR imaging were performed using a Varian millipede ^{1}H coil (Ø$_i$=40mm, 5cm length).

In vivo MR acquisitions Each animal underwent 4 MR sessions at day 0 (D0), 3 (D3), 5 (D5) and 7 (D7). For all sessions, mice were anesthetized using isoflurane (3% in O_2, 1.5 L.min^{-1}) and positioned in the magnet using a custom built cradle. An optimized gradient echo (GE) sequence was used for high resolution imaging of the MPIO-labeled SC distribution (TE/TR=3.8/170ms, matrix 256x256, FOV=19.2x19.2mm, 75µm in-plane resolution, 200µm slice thickness, 20 slices, NT=40, Tacq=29min). Anatomical landmarks were used to insure the reproducibility of slice positioning between MR sessions. At D7, dynamic contrast enhanced (DCE) MR imaging was performed in addition to high resolution MRI in order to confirm the location of the tumor and its permeable vasculature. Briefly, a bolus dose of Magnevist® (4 µmol/kg in 200 µl of PBS, Bayer Healthcare, USA) was injected through a 27G catheter secured in the tail vein of the animal, and a GE sequence using the same slice orientation as high resolution imaging was used to detect tumor enhancement (TE/TR=1.31/37ms, FOV=19.2x19.2mm, matrix 128x128, slice thickness=1mm, NT=10, Tacq=47s). All high-resolution GE data sets were zero-filled to 512x512, all DCE data sets to 256x256.

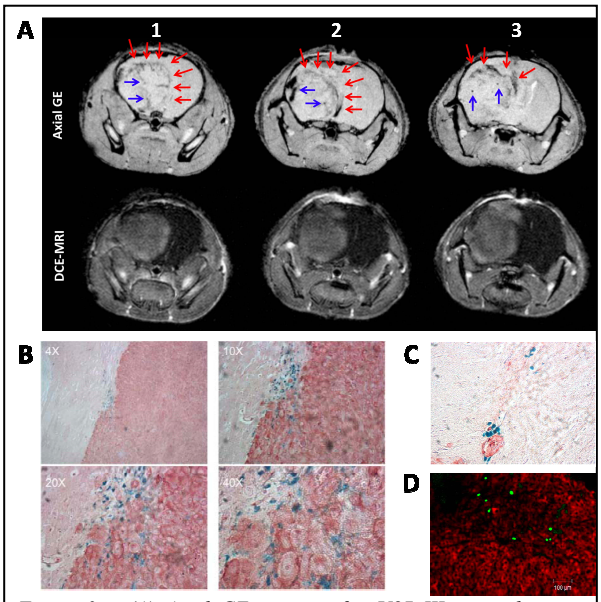

Figure 3 – (A) Axial GE images of a U87vIII tumor bearing mouse 7 days post injection of MPIO-labeled fNSCs (first row) and corresponding DCE-MRI images (second row). (B) Prussian blue (blue) and EGFR (brown, showing tumor) stainings confirm MPIO-labeled hMSC localizations at the tumor edge. (C) Tumor microsatellite surrounded by MPIO-labeled hMSCs. (D) BrdU labeled hMSCs located inside the tumor.

Histological analysis Euthanasia was performed immediately following the last imaging session at D7. All brains were resected, fixed in 10% buffered formalin, embedded in paraffin and sectioned along the imaging plane. Prussian blue staining was performed to allow detection of iron. EGFR staining was performed to identify tumor cells.

RESULTS & DISCUSSION

hMSCs and fNSCs were successfully labeled with MPIO particles, as illustrated in Figure 1. In all animals, for both hMSCs and fNSCs, areas of MPIO-induced hyposignals were seen to change during the 7-day observation period. Specifically, MPIO-labeled hMSCs and fNSCs were found to localize around the rim of the tumor and accumulate in this region between D3 and D7, as shown in Figures 2 and 3.A (red arrows). In some animals, MPIO-labeled hMSCs and fNSCs were also found in the tumor mass at the two latest time points (D5 and D7), as indicated by the blue arrows in Figures 2 and 3.A. Interestingly, areas of hyposignal generally co-localized with the edges of post-Gd enhancing regions, ie regions of high vascular permeability in tumor blood vessels, as shown in Figure 3.A. This observation is consistent with the report of SC tropism to brain tumors being induced by vascular endothelial growth factor (VEGF) (4). In order to confirm that the hyposignal is due to MPIO-labeled SCs, Prussian blue and EGFR histological stains were performed on excised tumors (Figure 3B and C). These reveal that the majority of MPIO-labeled SCs can be found at the edge of the tumor, and a smaller number found within the tumor mass. Interestingly, tumor microsatellites were found to be surrounded by MPIO-labeled SCs, as shown in Figure 3C. Importantly, these observations are consistent with data obtained from animals injected with BrdU-labeled SCs with no MPIOs (Figure 3D). In conclusion, MPIO-labeled hMSCs and fNSCs appear to behave in a similar fashion. First they localize to the rim of the tumor, in highly neovascularized regions then they can be found in the tumor mass and also further away tracking tumor microsatellites. Whereas an additional study using a slowly growing and highly invasive tumor type is underway to confirm these findings and possibly discriminate between hMSCs and fNSCs at longer time points, this study indicates that the tropism of both fNSC and MSC is comparable during a 7-day period of observation and thus either could be used in the development of SC-based therapies.

REFERENCES 1. James, Neuro Oncol (2009), 2. Saldanha, MRI (2010) 3. Hinds, Blood (2003) 4. Schmidt, Neoplasia (2005)

ACKNOWLEDGMENTS This work was supported by CIRM Disease Team grant DR1-01426 and by NCI grant CA097257.