FePro Labeled Splenocytes as Cellular Probe for MRI to Delineate the Margin, Early Detection of Recurrent or Metastatic Glioma

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Introduction: Glioma cell lysate-primed dendritic cells (antigen presenting cells, APC) have the potential to be potent vaccines against recurrent glioma by initiating a T-cell mediated cytotoxic immunity. Phase I and II clinical trials have been initiated (1). Primed dendritic cells initiate sensitization of T-cells against the glioma cells, which in turn migrate and attack the possible site of glioma and prevent early recurrence. Investigation showed large number of T-cells at the site of glioma when primed dendritic cells were used as vaccine (2). In the present study we hypothesize that splenocytes from glioma bearing host animals will recognize the glioma once they are re-injected in another tumor bearing recipient animal. T-cell and APC are abundant in the spleen. However, there is no imaging modality to detect the migration of the sensitized cells to the site of tumor without using labeling technique. Recently we have developed a technique to magnetically label cells using two FDA approved agents (ferumoxides and protamine sulfate) (3). We have shown that the migration and homing of magnetically labeled cells can be tracked by MRI. In this study we have used ferumoxides-protamine sulfate (FePro) labeled splenocytes from both control and tumor bearing host rats and re-injected the labeled cells into recipient rats bearing tumors to measure whether these labeled splenocytes delineate the margin of the tumors in recipients.

Methods: Rats was inoculated with 5000 gliosarcoma cells (9L) intracranially at 2 mm right of bregma, 2.5 mm anterior to bregma and 3mm deep into the brain. On day 14 after inoculation spleens from tumor bearing rats as well as spleens from control rats were acquired, homogenized and splenocytes were collected. All procedures were in accordance with our approved IACUC protocol. Splenocytes were labeled with FePro and re-injected in tumor bearing rats. Splenocytes from a single rat were re-injected in two rats. *In vivo* T1-weighted [TR/TE=500/10 ms, 128x128 matrix, 13-15 slices, 1 mm thick, 32 mm field of view (FOV), number of excitation (NEX)=4], T2-weighted [two-dimensional Fourier transformation (2DTF) multislice (13-15) multiecho (6 echoes), 32 mm FOV, 1 mm slice thickness, 128x128 matrix, and NEX = 2], T2*-weighted [multislice (13-15) multi gradient echo (6 echoes), 32 mm FOV, 1 mm slice thickness, 128x128 matrix, and NEX = 2] and 3D gradient echo (TR=100 msec, TE=6 msec, 10º of flip angle (FA), 32x32x16 mm^3 FOV, 256x192x64 matrix, and NEX = 1) images were obtained by a 7 Tesla magnetic resonance (MR) system at 3, 24 and 72 hours after injection. After 72 hours images, rats were euthanized, and the brain with tumors were sectioned, processed and stained for histological analysis to delineate tumor and Prussian blue for the presence of iron and the detection of labeled cells.

Results: Migration and homing of labeled splenocytes (splenocytes from tumor bearing rats) were detected as low signal intensity on MRI as early as 24 hours along the margin of the tumor. The low signal intensity rim was clearly visualized along the margin of the tumor at 72 hours on *in vivo* MRI and the tumor could be delineated from the surrounding brain tissues. In contrast, labeled splenocytes from control host rats without tumors did not migrate or home along the margin of the tumor and tumor could not be delineated from the surrounding normal brain tissues on MRI, however, some focal areas of low signal intensity were seen on MRI, which seems to be away from the tumor. Prussian blue staining of the histology sections showed iron positive cells along the margin of the tumor that received FePro labeled splenocytes from tumor bearing rats (Fig 1). No definite iron positive cell was seen along the margin of the tumor that received labeled splenocytes from control rats. Of note, a few metastatic spots away from the main tumor showed iron positive cells along the margin of the metastatic tumors, which corresponded to the low signal intensity on MRI.

Conclusion: Tumor cell sensitized cells (such as T-cells, APC or others) can be used as cellular probe to detect the margin of the tumor or early detection of metastatic or recurrent tumor. The technique may help in managing glioma patient by using their own peripheral T-cells or using cell-lysate-pulsed dendritic cell sensitized T-cells. FePro labeled sensitized T-cells can be used to delineate the margin or early detection of recurrent glioma.

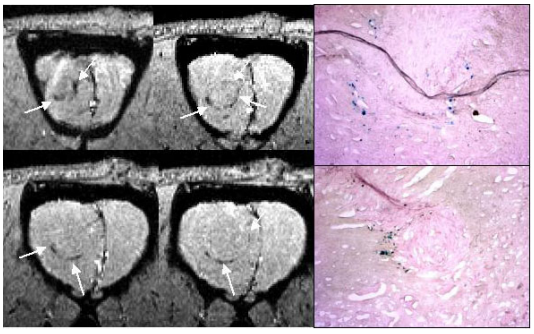

Fig 1: *In vivo* MRI and Prussian blue staining of rat brain with tumor that received labeled splenocytes from tumor bearing rat. Note the circular low signal intensity and iron positive cells along the margin of the tumor.

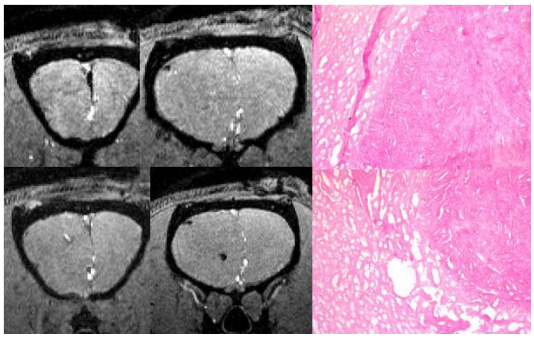

Fig 2: *In vivo* MRI and Prussian blue staining of rat brain with tumor that received labeled splenocytes from control rat. No iron positive cell is seen.

References: 1) Yamanaka R, et al. Br J Cancer. 2003; 89:1172-9, 2) Yu JS, et al. Cancer Res. 2001; 61:842-7, 3) Arbab AS, et al. Blood 2004; 104: 1217-1223.