Cellular Imaging of tumor infiltration by lymphocytes labeled with superparamagnetic particles : a comparative study at 7 and 9.4 T

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INTRODUCTION

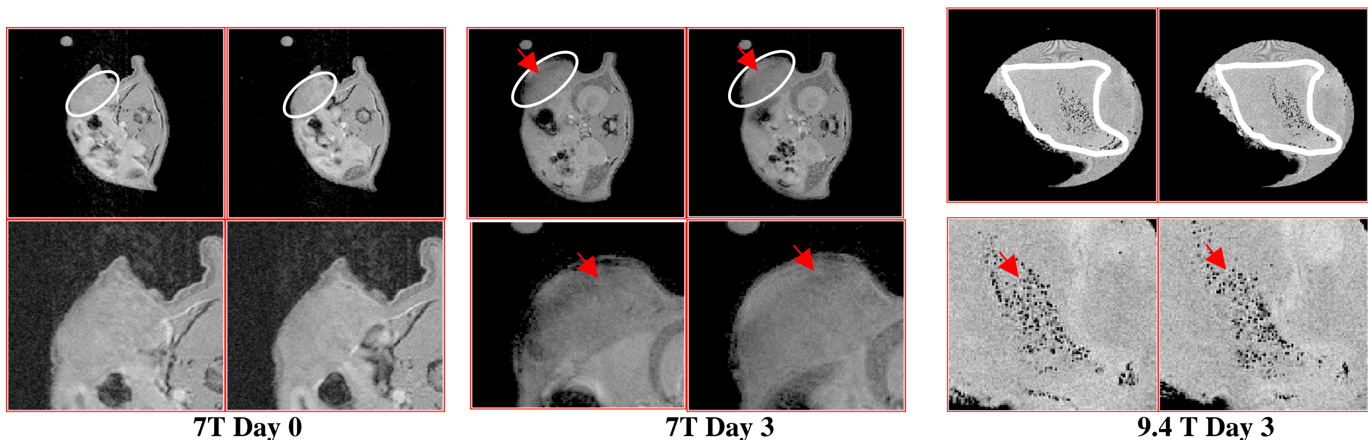
The aim of this study was the follow up by MRI of lymphocyte infiltration in implanted tumors in mice, and to compare the images obtained in vivo at 7T with those ex vivo at 9.4 T.

MATERIALS & METHODS

The model used was derived from an EL-4 lymphoma expressing the ovalbumin antigen, EG-7 cells. Lymphocytes expressing the T cell receptor (TCR) specific for ovalbumin were obtained from OT-1 mice, and were used as a cell model. Lymphocytes were in vitro activated (ConA and IL2) and labeled with anionic superparamagnetic particles by simple incubation (1.5 pg Fe per cell) (1,2,3) , then fluorescently labeled with CFSE. Anionic particles are composed of a maghemite core („-Fe2O3) with anionic citrate molecules adsorbed on the surface. Labeled cells were intravenously injected (5x106 retro orbital) in C57BL/6 mice bearing the EG7 tumors. Animals were imaged at different times post injection, (Day 1, 2, 3) on a 7 T. magnet, using gradient echo sequences (500/3,2/60°) and (360/10/60°) with a 3.5 cm FOV and a 0.5 mm slice thickness. Animals were then sacrificed, tumors harvested, then analyzed ex vivo by micro-MRI at 9.4 T with FOV = 0.9 x 0.9 x 0.9 cm, a matrix size = 512 x 128 x 256 (TR=500ms, TE=15ms). Iron quantification was performed by magnetophoresis (4), electron paramagnetic resonance. Flow cytometry was used to quantify the labeled cells inside the tumor.

RESULTS

Before injection of the labeled cells, tumors showed homogeneous signal on every sequences. Images showed hypointense signal zones in the tumor at Day 3 (Fig), suggesting the presence of labeled cells. Enhancement measurements showed a decrease of –26% ± 1,4 % to –46,5 ± 3 % depending on the parameters of the GRE sequences used. At Day 1, results did not show any significant enhancement of the tumor, but a negative spleen enhancement of –17,8 ± 7,3 %, suggesting the presence of labeled cells in the spleen. At 9.4T MRI (Fig), images at Day 3 showed distinct hypointense areas, compared to control tumors. Flow cytometry showed significant percentages of CD4 and CD8 lymphocytes which exhibited poor fluorescent labeling. Additional experiments are under way to quantify the cell infiltration in the tumor.



7T D0 : Preinjection axial image (GRE sequence TR/TE/angle) showing homogeneous signal
7T D3 : Same animal, 3 days after injection of labeled lymphocytes. An hypointense aera is clearly seen in the tumor.
9.4 T D3: Ex vivo , high resolution image Same tumor subsequently imaged at 9.4 T : Dark spots are evidenced in the tumor, indicating lymphocyte infiltration..

CONCLUSION

This study shows that visualization of lymphocytes migration into the tumor is feasible after intravenous injection of the cells, with a good correlation between in vivo and ex vivo results. Further studies are in progress to quantify the number of cells detectable at the different fields.

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