MRI of Angiogenesis in Xenograft Tumors Using Ferumoxides Labeled Sca1+ Cells

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The growth and metastasis of the majority of tumors depends on the formation of new blood vessels or angiogenesis. Emerging evidence indicates that bone marrow derived circulating endothelial progenitor cells (EPCs) can contribute to the angiogenesis. Until recently there was no direct method to image endothelial cells into neovasculature of tumors by MRI. In this study we tried to detect angiogenesis in a subcutaneous mouse flank tumor model using dual labeled mouse Sca1+ (mouse EPC, Ferumoxides and Fluorescent marker) either by intravenous administration of labeled Sca1+ cells or by implanting mixture of tumor cells with labeled Sca1+ cells.

Methods: Sca1+ cells were collected from the bone marrow of Swiss Webster mice and grown in stemspan media including proper cytokines for 3-7 days. Sca1+ cells were labeled with ferumoxides-poly-l-lysine complexes (ratio 1µg/ml:0.03 µg/ml), washed, double labeled with fluorescent dye DiI and injected intravenously (IV) in C6 tumor cell line implanted SCID mice (group 1), and subcutaneously mixed with tumor cells (group 2) and compared to control mice (group (3) that received unlabeled Sca1+ cells IV in mice with subcutaneously implanted tumor cells. For Group 1 and 3 animals tumor cells implantation ($1x10^6$ C6 cells) and IV injection of labeled cells ($2x10^6$ labeled Sca1+ cells) were done on the same day. For group 2 mice, $1x10^5$ labeled Sca1+ cells were mixed with $9x10^5$ tumor cells and implanted in flank of mice. MR imaging was performed at different stages of tumor growth from 0.5 cm to 2 cm in sizes. After in-vivo MRI, mice were euthanized and perfused with 4% paraformaldehyde, and tumors were collected for high-resolution ex-vivo MRI followed by fluorescent microscopy (for dye DiI), Prussian blue and immunohistological staining. In vivo MR images were acquired using a 7 T, 22 cm horizontal bore MR unit (Bruker, Billerica, Mass) with 39G/cm gradients and a 35 mm transmit-receive birdcage volume coil. In vivo MRI was performed using 2D T2* weighted gradient echo (TR/TE= 500-700/4.3 ms, NEX=8, slice thickness = 0.5 mm, matrix 384x512 and FOV = 2.6x2.6 cm), 2D T2 RARE (TR/TE=2400/7 ms; rare factor = 8, NEX=8, matrix 256x256, FOV 2.6x2.6 cm). Ex vivo MRI was performed using 3D GRE imaging (TR/TE=270/4.3 ms, NEX=4). Resolution in 2D images was 500x100x100 to 500x80x70 microns and in 3D was 105x105x60 micron.

Results: Gradient echo images showed multiple hypointensities (Fig. 1) in the tumor in group 1 mice when tumor size reached about 2 cm and the pattern and intensity of hypointensity was different from that of control mice (group 3). For group 2 mice, whole tumor was hypointense on gradient echo or T2-weighted images at tumor size of less than 1 cm but the hypointensity became linear when the tumor size reached $\geq$1.5 cm. On ex-vivo MR images of perfused tumors, the hypointensity signals disappeared from control group (group 3) but remained in group 1 and group 2. Prussian blue staining showed abundant iron positive cells in the tumor of both group 1 and group 2 mice.

Conclusion: Detection of magnetically labeled EPC in implanted flank tumor vasculature was observed on in vivo and ex vivo MRI.

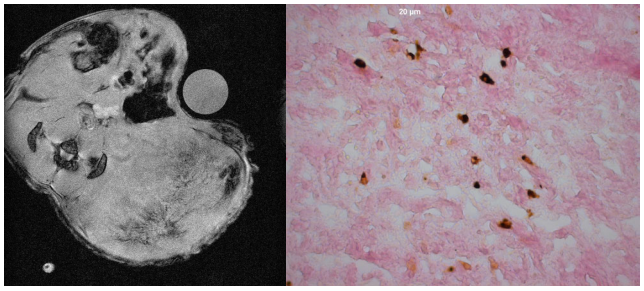

Fig. 1. In vivo MRI of a group 1 mouse at tumor size of 1.5 cm (11 days) and corresponding DAB enhanced Prussian blue staining showed multiple iron positive cells in the tumor.