Monitoring angiogenesis at microscopic levels: comparison between high resolution MRI and optical microscopy

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Purpose/Introduction: To provide an extended perspective to the assessment of angiogenesis by direct correlation between optical and intravital (fluorescence) microscopy and high resolution magnetic resonance imaging (MRI).

Subjects and Methods: 6 rats were prepared with a MRI-compatible dorsal skin-fold window chamber. Rats were anesthetized and hair removed from their backs. A 2x 12-mm diameter flap of skin is dissected away, leaving the fascia, and sandwiched between two frames and fixed with sutures. On both sides the windows are closed with a 12-mm diameter microscopic cover glass of 0.13-0.16 mm thickness. Before closing of the surgical site a small piece of tumor (0.1 mm^3) is transplanted in the fascia using a micro-surgical microscope. The tumor was a high grade, highly vascularized, non-immunogenic BN-175 soft tissue sarcoma. MRI was then performed one week after the implantation procedure for 5 sessions in a 2 week time period. For each session, Albumin-$(gadopentetate)_{35}$, a macromolecular MR blood pool contrast agent, was first utilized to visualize blood and the vascular density around the growing tumor. Magnetivist (Gd-DTPA, Schering, Germany) was used subsequently to help delineate the tumor mass. For high resolution MRI, a 2 cm loop receiver was built and positioned over the window and a 3.0 T scanner (General Electric, USA) utilized for imaging at 72 x 72 x 100 μm^3 voxels (~16-18 min, 3D SPGR with TR/TE/angle=28/4.8 ms,30^o – 54-64 slices). A 1 cm loop was used in particular occasions to obtain higher in-plane resolution down to 50 x 50 μm^2. Intravital and optical microscopy, and macro digital photography were performed for correlation. MRI data were volume rendered using Voxelview (Vital Images, Minneapolis, USA), to illustrate spatial-temporal relationships of vascular growth/angiogenesis and tumor size information.

Results and discussion: Alb-Gd-$DTPA_{35}$ permitted an accurate delineation of microvessels, making it possible to demonstrate exquisitely the vascular network around the forming tumor. Figure 1 illustrates a 3 scans from a time series collected over a period of 12 days on an implanted tumor in the window. In many instances tumor visibility was not apparent using only the intravascular contrast agent because of slower leakage to the interstitium and tumurous tissue and/or decreased vascular density. Gd-DTPA leaving to the intersitium provided a high signal intensity around the tumor, helping delineate the tumor boundaries and provide \additional detail regarding tumor structure. Intravital microscopy confirmed a very heterogeneous distribution of systematically injected liposomes in the window. Optical microscopy and macro photography provided exceptional correlation to the vascular signal distribution detected by MRI (Figure 2).

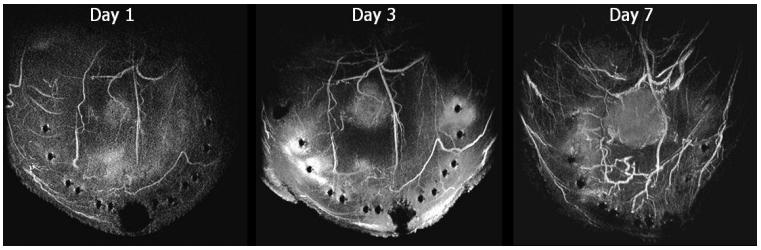

Figure 1: Time series of growing tumor implanted in the center of the window. Vascular density increases as the tumor develops, with a larger network of vessels feeding the boundary of the tumorous mass. Thin maximum intensity projections are show to better demonstrate the mass against the insterstitium.

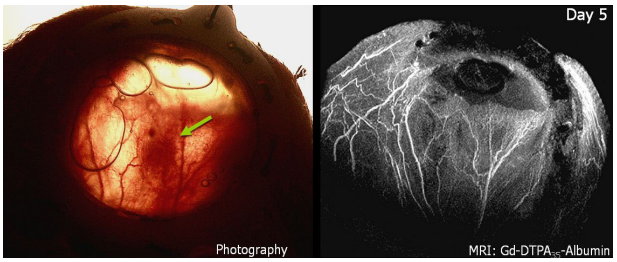

Figure 2: Optical photography using the macro setting on a Minolta Z2 digital camera compared to a maximum intensity projection of the MRI scan using the intravascular contrast agent. The thickness of the window containing the tumor at this stage was approximately 1.3 mm.

Conclusions: Mapping of the developing vascular tumor bed can be performed in-vivo with high resolution MRI with accurate correlation to optical and intravital microscopy. The window model provides a unique opportunity to precisely quantify the capabilities of diverse MRI contrast agents for the quantification of tumor permeability, parametrics describing angiogenesis and the effectiveness of anti-tumor therapy.