MR Imaging of Bone Marrow-Derived Mesenchymal Stem Cells Incorporation into Tumor Vasculature in Prostate Cancer Models

L. Ostrovskaya[1], Y. Kato[1], P. Walczak[1], B. Okollie[1], J. W. Bulte[2,3], D. Artemov[1]

[1]Johns Hopkins University, Baltimore, Maryland, United States, [2]Radiology, Johns Hopkins University, Baltimore, MD, United States, [3]Institute for Cell Engineering, Johns Hopkins University, Baltimore, Maryland, United States

Introduction: Solid tumors require neovascularization for their growth and progression. Recent data suggest that circulating bone marrow–derived mesenchymal stem cells (MSCs) play a significant role in tumor vasculogenesis. Development of reliable in vivo techniques to detect tumor vasculature and to identify bone marrow-derived endothelial stem cells in the functional neovasculature is necessary for detailed studies of tumor progression and for testing various anti-angiogenic therapies. We used noninvasive MRI technology to monitor homing and progression of MSCs in the tumor neovasculature in mouse prostate cancer models.

Methods: We monitored, with MRI, the incorporation of MSCs labeled with iron-oxide particles within the vasculature of mouse prostate cancer models with significantly different growth rates: highly aggressive MatLyLu prostate cancer and slow growing human PC-3 prostate cancer. For intravenous injection we used established mouse mesenchymal stem cell line developed by the group of Dr.Prockop, Tulane University. These cells were originally derived from femurs and tibiae of C57B1 mice with constitutively expressed GFP. Differentiation assay performed with Passage 5 cells demonstrate that they maintain pluripotency. MSCs transiently labeled *in vitro* with iron-oxide Bangs particles (diameter 0.9 μm) were detected by MRI *in vivo* in prostate cancer models. Briefly, PC3 and MatLyLu tumor cells were inoculated subcutaneously in male SCID mice. When first signs of tumor growth appeared, MSCs in suspension were slowly injected to the tail vein of the animal (1 million in 100 μl Saline). Animals were imaged with T2-weighted MRI at 9.4T animal horizontal-bore Bruker Biospec scanner before, during 3 days post injection, and then every week. MRI images of the tumors were obtained with home-built mouse body coil or a single turn resonator wrapped around the tumor. MR angiography of the tumor was performed using high molecular weight contrast agent, AlbuminGdDTPA. T1 weighted 3D fast spin-echo acquisition was performed before and after administration of the contrast.

Results and Discussion: A significantly higher initial accumulation of MSCs was detected in highly aggressive MatLyLu prostate cancer in comparison to slow growing human PC-3 prostate cancer model as detected *in vivo* (Fig.1) and *ex vivo* (Fig.2). Possible reasons for these experimental data can be related to variable aggressiveness, growth rate, and microenvironmental milieu resulting in different dynamics of vascular development. Thus, one plausible explanation is that aggressive rapidly growing tumors depend to a greater extent upon the recruitment of MSCs to form functional vasculature to sustain tumor growth. Indeed, highly aggressive MatLyLu prostate cancer is characterized by intense vascularization as shown in Fig. 3. MR angiography demonstrates spatial architecture of the tumor vasculature and can be used to correlate MSCs homing to the tumor blood vessels.

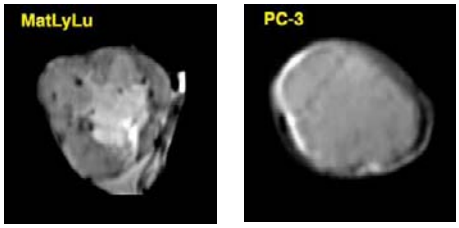

Figure 1. T2-weighted MR imaging MatLyLu and PC-3 tumors in mice injected with iron-oxide labeled MSCs. Dark spots in the images correspond to the position of MSCs in the tumor**.**

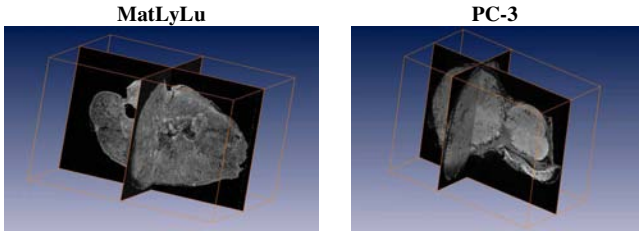

Figure 2. 3D reconstruction of MR images of excited MatLyLu and PC-3 tumors obtained *ex vivo* with T2-weighted fast spin-echo sequence (RARE, TE/TR= 57/2000 ms, ETL=8)

Figure 3. 3D MR angiography reconstruction of the vascular tree in MatLyLu tumor.

Conclusions: Mesenchymal stem cells play a significant role in tumor neovascularization, and it is possible to use noninvasive MRI technology to monitor homing and progression of MSCs in the tumor neovasculature in prostate cancer models. Although the *in vitro* labeling protocol resulted in efficient transient cell labeling for MRI, it might not be feasible for long-term monitoring of MSCs in the tumor vasculature potentially due to dilution of the label. Short-term labeling/imaging of MSCs homing also does not provide information about the eventual fate of these cells and, in fact, may only represent the trapping of circulating cells by the tortuous and hyper-permeable tumor vasculature. Novel strategies that enable long term monitoring of MSCs are needed to follow the dynamics of their incorporation and function in the tumor neovasculature.