Migration of SPIO Labled EPCs and Angiogenic Factors

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Introduction: Previously we have reported the migration and incorporation of magnetically labeled endothelial progenitor cells (EPCs) into neovasculatures of implanted tumors (1,2). Both VEGF and HIF-α have been said to be related to angiogenesis in tumors. However, recent finding showed SDF-1 is a strong chemo-attractant for migration of EPCs or hematopoietic stem cells (HSCs). The purpose of the study was to determine which factor is responsible for the migration and homing of magnetically labeled endothelial progenitor cells (EPCs) at the sites of active tumor angiogenesis.

Methods: Three tumor models were used. 1) Magnetically labeled EPCs were administered intravenously in nude mice bearing 0.2 cm rat glioma in right flank; 2) magnetically labeled EPCs were mixed with rat glioma tumor cells and implanted in flank of nude mice; 3) magnetically labeled EPCs were mixed with human melanoma tumor cells and implanted in flank of nude mice. EPCs (AC133+ cells) were obtained from healthy volunteers or from cord blood. EPCs were labeled with ferumoxides-protamine sulfate complexes. Both *in vivo* and *ex vivo* MRIs were obtained using 7 Tesla MR System. *In vivo* T2W, T2*W and 3D GRE images were obtained with following parameters. T2WI: Multi-echo (6 echo), multislice, 32 mm FOV, 1 mm slice thickness, 128x128 to 256x256 matrix, and NEX = 2. T2*W: Multislice (13-15) multi gradient echo (6 echoes), 32 mm FOV, 1 mm slice thickness, 128x128 to 256x256 matrix, and NEX = 2. 3D gradient echo: TR=100 msec, TE=6 msec, 10º of flip angle (FA), 32x32x16 mm3 FOV, 256x192x64 matrix, and NEX = 1. *Ex vivo* 3D gradient echo MRI was performed with TR=200, TE=10, FA=10, NEX=4, FOV= 3.2x2.0x10, Matrix=512x312x160 which produces an image resolution of 62.5x62.5x62.5 microns3. Animals were perfused and the tumors (0.5- 1.5 cm) with surrounding tissues were collected. Serial sections of the tumor were made and consecutive sections were stained for DAB enhanced Prussian blue (PB), platelet derived growth factor (PDGF), hypoxia inducible factor 1α (HIF-1α), stromal derived factor 1 (SDF-1), matrix metalloproteinase 2 (MMP-2), VEGF and endothelial markers such as CD31 and vWF.

Results: MRI demonstrated hypointensity regions at the periphery of the tumors where the PB^+-EPCs were positive for endothelial cell markers. At sites of PB^+-EPCs, both HIF-1α and SDF-1 were strongly positive and PDGF and MMP-2 showed generalized expression in the tumor and surrounding tissues. Tumors cells expressed VEGF however no strong expression of VEGF associated with PB^+-EPCs was seen.

Conclusion: Labeled EPCs' migration seemed to be related to HIF-1α and SDF-1 expression. SPIO labeled cells can be used as probes for MRI in cell migration studies. SPIO labeled cells can be used as marker for histological identification of administered cells similar to that of fluorescent dyes or reporter genes. Tracking of SPIO labeled cell can help real time MRI guided biopsy for molecular analysis in different cell migration studies.

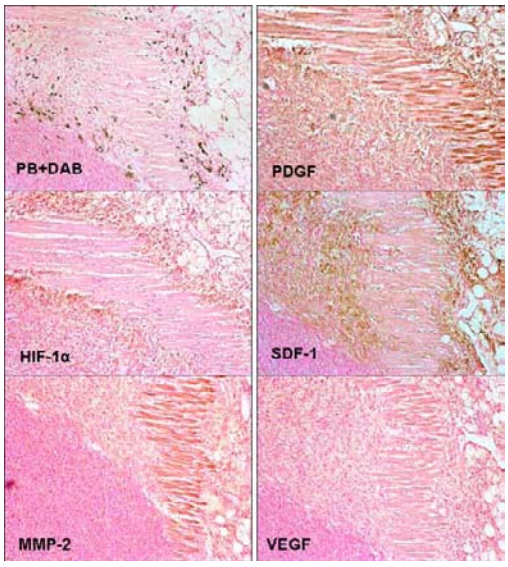

Figure 1: Expression of different angiogenic and chemo attractant factors at the sites of migrated labeled EPCs in tumors (consecutive sections).

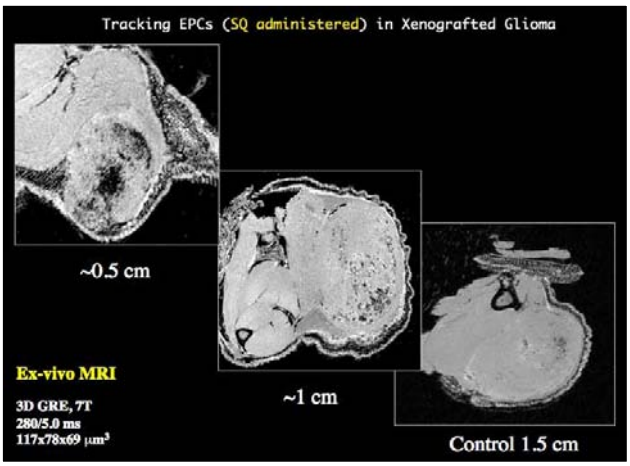

Figure 2: Migration of locally implanted magnetically labeled EPCs depicted by ex vivo MRI. Low signal intensity due to labeled EPCs were mostly seen at the center of the tumor when the size of the tumor was around 0.5 cm, however, the low signal intensity was seen at the periphery when tumor size was more than 1 cm. Please note the low signal intensity seen in control tumor due to necrosis.

References: *1. Anderson et al. Blood 2005, 2. Arbab et al, Stem Cells, 2006.*