Magnetically labeled transgenic endothelial progenitor and dendritic cells as probes for cellular MRI and gene delivery vehicles

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INTRODUCTION: Gene therapy holds enormous therapeutic potential for breast cancer treatment. However, the infiltrative nature of breast cancer poses a problem for successful delivery of needed genes to the sites of invading tumor cells, when administered locally. In addition, several factors, including lack of an efficient vector and delivery system itself, limit the effectiveness of systemically delivered genes. Very recently, stem/progenitor cells have been considered as delivery vehicles for transferring exogenous genes to the cancer cells. It is utmost necessary to monitor the migration and homing of the genetically modified administered cells. However, current *in vivo* imaging techniques lack the ability to track the real time migration and homing of genetically altered cells with acceptable resolution to the cancer tissue. The purpose of this study was to determine whether endothelial progenitor (EPCs) and/or dendritic cells (DCs) can be used as gene delivery vehicles and as cellular probes for magnetic resonance imaging (MRI). In this study, we used superparamagentic iron oxide (SPIO)-labeled endothelial progenitor cells (EPCs) and dendritic cells (DCs) to carry human sodium iodide symporter (hNIS) gene to the sites of implanted breast cancer in mouse model. *In vivo* real time tracking of these cells was performed by magnetic resonance imaging (MRI) and expression of hNIS was determined by Tc-99m pertechnetate (Tc-99m) scan.

METHODS: Three million human breast cancer (MDA-MB-231) cells in 50 µl of serum free media were subcutaneously implanted in the right flank of nude mice. EPCs were isolated from fresh human cord blood. Dendritic cells were differentiated from the cord blood CD34+ or CD14+ cells. Both EPCs and DCs were genetically transformed to carry hNIS gene using adenoviral vectors. Both control and genetically transformed EPCs and DCs were magnetically labeled with ferumoxides-protamine sulfate (FePro) complexes as previously described (1). To confirm transfection, technetium-99m pertechnetate (Tc-99m) uptake study was performed and the radioactivity was determined by a gamma counter. Mean intracellular iron was determined by UV/VIS spectrophotometric method using hydrochloric acid and potassium ferrocyanide. Magnetically labeled control, genetically transformed, and unlabeled cells were administered intravenously in tumor bearing mice when tumors grew to 0.5 cm in sizes. MRIs were acquired 7 days after cell injection and SPECT images were acquired within the 24 hours of performing the MRI. T2, T2* and 3D GRE images were obtained using a 7 Tesla, 20 cm bore superconducting magnet (Magnex Scientific, Abingdon, England) interfaced to a BRUKER console (Bellerica, MA). SPECT images were acquired with custom built micro-SPECT (converted from a clinical PRISM 3000XP using multi-pinhole animal collimators from Bioscan Inc.) using Tc-99m. After SPECT, animals were euthanized, perfused and whole tumors were collected for the *ex vivo* measurement of radioactivity, and histochemical determination of iron labeled cells using Prussian blue staining. The presence of human cells was also determined by staining with anti-human CD45 antibody. Expression of hNIS in accumulated cells was determined by staining with anti-hNIS antibody.

RESULTS: Both cell lines were efficiently labeled with ferumoxides-protamine sulfate (FePro) complexes. Labeling efficiency was more than 90%, as determined by manual counting of PB-stained and unstained cells using a microscope. Transfected cells exhibited significantly increased radioactivity as compared to the non-transfected cells. MRI images clearly showed the presence of low signal intensity areas around the tumors in mice that received iron labeled cells, indicating the accumulation of administered cells. The presence of iron labeled cells was also confirmed by Prussian blue staining (Fig 1). In addition, SPECT images showed significantly higher radioactivity in tumors (compared to background activity) in animals that received transfected cells, and no significantly higher radioactivity was observed in tumors in animals that received non-transfected cells (Fig 1). Presence of administered cells was also confirmed by the presence of human CD45 and hNIS positive cells.

CONCLUSION: Both, MRI and SPECT images showed accumulation of administered EPCs and DCs in implanted breast cancer, and expression of hNIS gene, respectively. Our study indicates that both EPCs and DCs can be used to deliver genes by systemic administration. Genetically transformed, magnetically labeled DCs or EPCs can be used both as delivery vehicles and as cellular probes for detecting *in vivo* migration and homing of cells by MRI. This method can be used in the future development of gene therapy approaches where genetically modified cells can be tracked by real time *in vivo* MR scanning in different disease processes.

References: 1) Arbab et. al. Blood; 2004.

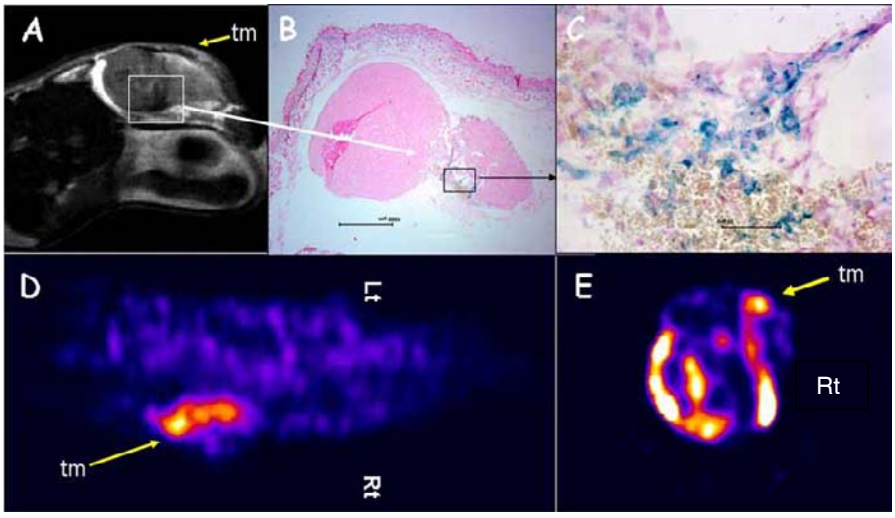

Figure 1 shows the T2WI (TR/TE = 3000/40 ms) (**A**), corresponding Prussian blue staining (**B, C**) and images of Tc-99m SPECT (**D, E**) in breast cancer bearing mouse that received magnetically labeled transgenic mature DCs. Axial MRI (1 mm thick) shows low signal intensity area (within rectangle in **A**) in the tumor. Prussian blue staining (**B** = x2, **C** = x40) shows iron positive cells at the corresponding sites of low signal intensity areas observed on MRI. Coronal (**D**, 5 mm thick) and axial (**E**, 0.8 mm thick) sections of SPECT images show intense activity of Tc-99m at the corresponding site of tumor. tm=tumor, Lt=left, Rt=right.