Use of Endothelial Progenitor Cells as Gene Carrier and Multimodal Imaging Probes

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Purposes: The purpose of the study was to determine whether intravenously or locally administered genetically transformed and/or magnetically labeled cord blood derived endothelial progenitor cells (EPCs) can simultaneously play a role as a gene carrier system for human sodium iodide symporter (hNIS) and as imaging probe for magnetic resonance imaging (MRI) and/or single photon emission computed tomography (SPECT) in a rat model of human glioma.

Materials and Methods: *Cells:* EPCs were collected from human cord blood with an approved IRB. The collated EPCs were cultured for 10-15 days and transduced to carry hNIS by either lentiviral or replication competent adenoviral vector. Cellular viability and transduction efficiency (Tc-99 uptake assay) were determined following magnetic labeling with ferumoxides-protamine sulfate (FePro). Human glioma cell line (U251) was used to create orthotopic tumor. *Animal model:* Athymic nude rat 6-8 weeks of age and 150-170g of weight (Charles River Laboratory, Inc.) was used to make glioma using U251 cells and stereotactic techniques. *Intravenous administration of EPCs:* Five to ten millions EPCs were intravenously administered in rats bearing human glioma though tail vein after 14 days of implantation. All animals underwent pre-injection MRI. Five different groups of animals received different doses of cells as follows: 1) $5x10^6$ non-labeled non-transgenic (control) EPCs, 2) $5x10^6$ non-labeled but transgenic EPCs, 3) $5x10^6$ labeled non-transgenic EPCs, 4) $5x10^6$ labeled transgenic EPCs, and 5) $5x10^6$ labeled transgenic plus $5x10^6$ non-labeled but transgenic EPCs. Upon administration of EPCs, rats underwent MRI and Tc-99m single photon emission computed tomography (SPECT) scanning to determine the migration and accumulation of FePro labeled and transgenic (containing hNIS) cells, respectively. *Local administration of EPCs:* One to two million EPCs was administered either in the tumors or in contralateral hemisphere in rats bearing human glioma after 14 days of tumor implantation. Pre-injection MRI was performed on all animals. Six different groups of animals received different doses of cells as follows; 1) intratumor inoculation of $1x10^6$ labeled non-transgenic (control) EPCs, 2) intratumor inoculation of $1x10^6$ labeled and transgenic EPCs, 3) intratumor inoculation of $1x10^6$ non-labeled and transgenic and $1x10^6$ labeled transgenic EPCs, 4) contralateral inoculation of $1x10^6$ non-labeled and transgenic and $1x10^6$ labeled transgenic EPCs, 5) intratumor inoculation of $1x10^6$ non-labeled transgenic followed by repeated SPECT for two weeks, and 6) intratumor inoculation of $1x10^6$ non-labeled non-transgenic followed by repeated SPECT for two weeks. Following administration of EPCs, rats underwent MRI and Tc-99m SPECT scanning to determine the migration and accumulation of FePro labeled and transgenic (containing hNIS) cells, respectively. *Imaging Studies:* MR images were obtained with a 3.0 Tesla clinical system (Signa Excite, GE health) using 50 mm diameter x 108 mm RF rung length small animal imaging coil (Litzcage small animal imaging system, Doty Scientific Inc, Columbia, SC). T2*-weighted and 3D FIESTA images of the tumor bearing brain were acquired. SPECT images were acquired with a dedicated PRISM 3000 gamma camera fitted with multi-pinhole rat collimator, 360 degree rotation with 36 degree increments, 180 sec per projection, using 256x256 matrices with a field of view of 4x6 cm. *Histological Analysis:* Immediately after SPECT imaging, animals were euthanized and perfused with saline and paraformaldehyde in order to dissect and further analyze tissues by histology. Prussian blue (PB) and expression of hNIS in accumulated cells was determined.

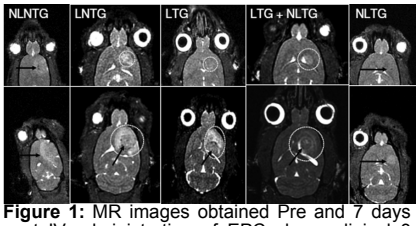

Figure 1: MR images obtained Pre and 7 days post IV administration of EPCs by a clinical 3 Tesla MRI system using FIESTA sequence. Note the low signal intensity areas in and around the tumors that received magnetically labeled cells. NLNTG= non-magnetically labeled, non-transgenic EPCs, LNTG= magnetically labeled non-transgenic EPCs, LTG= magnetically labeled transgenic EPCs, and NLTG= non-magnetically labeled transgenic EPCs.

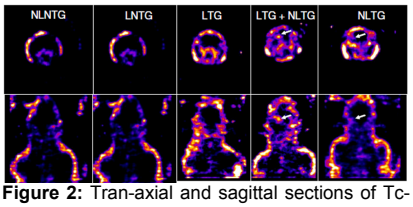

Figure 2: Tran-axial and sagittal sections of Tc-99m SPECT images obtained 8 days post IV administration of EPCs.

Results: Magnetic labeling caused around 20% decrease in Tc-99 uptake in transduced cells; however, EPCs expressed hNIS (detected by Tc-99m SPECT) in tumors that received transduced cells either by local or IV administration. Activity of Tc-99m was also dependent on the number of EPC administered. More activity was detected in tumors that received double doses of transduced EPCs. On the other hand MRI was able to determine the migration and accumulation of IV administered magnetically labeled EPCs even with lower doses. Direct inoculation of EPCs showed large area of low signal intensity areas at the site of injection of magnetically labeled EPCs. There was slight migration of low signal intensity observed after 7 days on MRI when magnetically labeled EPCs were injected into tumor. Immunohistochemical analysis showed both PB and hNIS positive cells in the tumors. Figures 1 and 2 show MRI and SPECT images from representative cases with IV injections.

Conclusion: Cord blood derived EPCs were able to carry and express hNIS in glioma when administered both, locally and intravenously. Magnetic labeling allowed detection of EPCs' migration and accumulation in tumor by MRI. SPECT also detected migration of EPCs and expression of hNIS gene. EPCs can be used as imaging probes and gene carrier/delivery system for glioma.