SPIO-Labeled Natural Killer Cells: Cytotoxicity and In Vivo Imaging

C. L. Mallett[1,2], C. Ramsay[1], and P. J. Foster[1,2]
[1]Imaging Research Laboratories, Robarts Research Institute, London, Ontario, Canada, [2]Medical Biophysics, The University of Western Ontario, London, Ontario, Canada

Introduction: Natural killer (NK) cells are a type of lymphocyte that play a key role in the cell-based immune defense against virus-infected and malignant cells. Cancer therapy with NK cells can be performed by the administration of *ex vivo* expanded and activated donor cells, which are highly toxic against malignant cells without affecting normal cells. Many important questions persist, however, relating to the spatial and temporal migration of NK cells after their administration. Cellular MRI allows for the *in vivo* tracking of cells after their labeling with magnetic nanoparticles. In 2005, Daldrup-Link *et al.* (1) showed that NK-92 cells can be labeled with iron particles and detected *in vivo* in mammary tumors in mice. There have been no other similar publications since. This may be related to the fact that lymphocytes are among the most difficult cells to label with enough iron particles to allow them to be detected by MRI. In our lab we are interested in NK cell therapy for prostate cancer. Here we demonstrate that NK cells can be readily labeled with superparamagnetic iron oxide (SPIO) nanoparticles and that they maintain their cytotoxic effect on prostate cancer cells. We also show that these cells can be detected by MRI following subcutaneous injection into the flank of healthy nude mice and that their migration from the site of injection to the nearby popliteal lymph nodes can be tracked.

Methods: We used the human NK cell line KHYG-1 (2) and the human prostate cancer cell line PC-3M. The KHYG-1 cells were labeled with PKH67, a green fluorescent cell linker dye (Sigma) and with 100 µg Fe/mL of MoldayION-RhodamineB (Molday-Rho, BioPal) at a concentration of 2e6 cells per 4 mL by co-incubation for 24-36 hours. Viability after labeling was assessed by trypan blue exclusion. Labeling efficiency was assessed by examining cytospin slides stained with Perl's Prussian Blue (PPB); the percentage of labeled cells was determined by counting cells with and without blue staining. The distribution of Molday-Rho in KHYG-1 cells was also visualized by fluorescence imaging of the rhodamine B tag. To assess KHYG-1 induced cytotoxicity of PC-3M cells, 100 µL of PC-3M cells at 8e4/mL were plated in a 96-well plate and allowed to adhere overnight. Labeled and unlabeled KHYG-1 cells were then added at 3:1, 9:1 and 15:1 ratios of KHGY-1:PC-3M and co-cultured for 24 hours. Viability was assessed using the MTT assay. For *in vivo* MRI, 1 million and 0.5 million labeled KHYG-1 cells were injected subcutaneously into the right and left flank, respectively, of a male nude mouse, which was imaged that day and 9 days later. Scans were performed using clinical 1.5 and 3T GE systems with a custom-built high performance insert gradient and solenoidal RF coils. Steady state free precession (SSFP) images of the mouse body were acquired with 200 micron isotropic spatial resolution in less than 25 minutes.

Results: KHYG-1 cells were readily labeled with Molday-Rho by simple co-incubation at high viability (typically >90%) and labeling efficiency (80%) (Figure 1). Co-culture of KHYG-1 and PC-3M showed that at the lowest ratio of KHYG-1 to PC-3M (3:1), labeled cells were less cytotoxic; however, at higher ratios both labeled and unlabeled KHYG-1 were equally toxic (Figure 2). Labeled KHYG-1 were tracked *in vivo* over time after flank injection and appeared to migrate toward the popliteal lymph node (Figure 3).

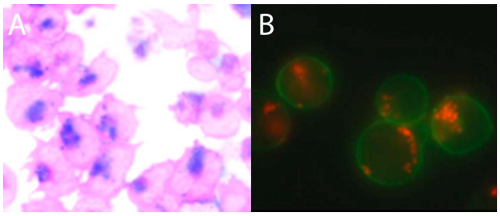


Figure 1: KHYG-1 cells labeled with Molday-Rho. A) PPB stained cytospin slide. Blue indicates iron. B) Fluorescent image. Green indicates cell membrane stained with PKH and red is from rhodamineB.

Figure 2: Effect of 24-hour co-culture with labeled and unlabeled KHYG-1 on PC-3M viability, normalized to viability of PC-3M cells in media.

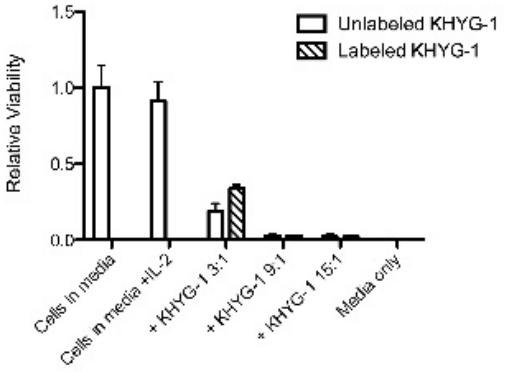
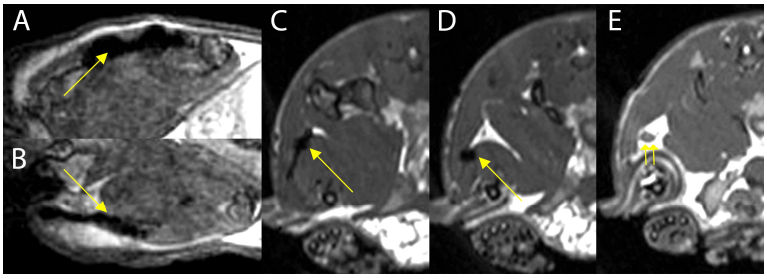


Figure 3: Injection and tracking of labeled KHYG-1 *in vivo*. A) and B) show right (1million cells) and left (0.5 million cells) flank at site of injection 2 hours after injection. (C-E): Axial reconstruction of coronally-acquired images shows that at day 9 post-injection the region of void has extended from the site of injection to the popliteal lymph node. Single arrows indicate regions of signal loss and double arrows point to the popliteal node.



Discussion: This is the first report of the labeling and imaging of KHYG-1 cells. KHYG-1 cells are unique as a NK cell type having enhanced cytotoxicity against cancer cells and therefore are a valuable model cell type for immunotherapy. Labeling was easily achieved by simple co-incubation and had high labeling efficiency and cell viability. The labeled KHYG-1 cells were cytotoxic against prostate cancer cells *in vitro*. Labeled KHYG-1 were tracked over 9 days moving toward the popliteal lymph node from the site of injection in the flank. These experiments set the stage for our future work, which will focus on tracking the location of KHYG-1 cells in mice with PC-3M-derived tumours. MR tracking of NK cells should improve our understanding of novel adoptive cell therapies for cancer.

References: 1. Daldrup-Link HE et al. *Eur Radiol.* 2005 Jan;15(1): 4–13
2. Yagita et al. Leukemia. 2000 14(5): 922-920