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

C. L. Mallett1,2, C. Ramsay1, and P. J. Foster1,2
1Imaging Research Laboratories, Robarts Research Institute, London, Ontario, Canada, 2Medical 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, KHYG-1 cells at 2e6/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 KHYG-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).

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.