Magnetic Resonance Imaging Tracking of Dendritic Cells Homing to the Draining Lymph Nodes in Mice

Ye Xu[1,2], Dan Wang[1], Yanhong Liu[1], Chunchao Xia[3], Qiyong Gong[3], Bin Song[3], Chuangqiang Wu[1], Gan Lin[1], Danyang Li[1], Hua Ai[1,3,*]

[1] National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, PR China
[2]Departemtent of Radiology, Chongqing Medical University, Chongqing, PR China
[3]Department of Radiology, West China Hospital, Sichuan University, Chengdu, PR China

Statement of Purpose: Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system. DC based cell therapies are currently approved or under clinical trials for treatment of cancer, diabetes and other diseases. Efficient delivery and subsequent migration of vaccinated DCs to regional lymph nodes is of great importance for effective stimulation of the immune system. However, monitoring of cell trafficking inside body is not feasible without imaging techniques. Magnetic resonance imaging (MRI) is a widely used imaging technology in clinical practice. DCs labeled with imaging probes provides opportunity for one to track them *in vivo* noninvasively and dynamically. In this study, we explore to monitor the migration of DCs *in vivo* by labeling with a MRI probe Alkyl-PEI2k/SPIO, which have been successfully used for labeling of stem cells and other kinds of cells.[1-3] Migration of DCs to the draining lymph nodes in mice was observed under 3T and 7T MR scanners.

Methods: N-alkyl-PEI2k stabilized superparamagnetic iron oxide (SPIO) nanoparticles were synthesized following a previous protocol[1] and conjugated with fluorescence tag FITC. BALB/c mice were obtained from the Center of Laboratory Animal of Sichuan University. All studies involving animals were approved by the institute's animal care and use committee. DCs were generated and induced from bone marrow cells of murine femurs and tibia following a reported procedure.[4] To label the cells, the SPIO nanocomposites were incubated with DCs at different concentrations overnight. After labeling, assays of labeling efficiency, cell viability and phenotypes were performed by Hoechst staining, confocal laser scanning microscopy (CLSM), flow cytometry, Perls' Prussian blue staining and transmission electron microscopy (TEM). MRI studies of SPIO-labeled murine DCs pellets were conducted to find the MRI sensitivity of the cell labeling. Finally, *in vivo* MRI under 3T and 7T scanners was performed for tracking of DCs homing to the draining lymph nodes.

Results: MRI probes Alkyl-PEI2k/SPIO conjugated with FITC can form stable nanocomposites in physiological buffers. Bone marrow-derived DCs (BM-DCs) were induced to differentiate *in vitro* with a yield of about 1×10^8 cells/mice. DCs can be directly labeled with SPIO nanocomposites after 24 h incubation, which were further confirmed by Perls' staining, CLSM and TEM (**Fig. 1**). There was no obvious morphological change between the labeled and unlabeled cells. Hoechst staining also revealed no apoptosis introduced by SPIO labeling. The colorimetric ferrozine assay showed the SPIO uptake process in DCs was a time- and dose-dependent behavior. The major surface markers were studied by flow cytometry and compared between labeled and unlabeled DCs. No significant difference was found between them. *In vitro* MRI demonstrated that T_2 values decreased with the increase of cell number. *In vivo* MRI were performed under 3T and 7T scanners respectively after injection of titration numbers of DCs into the footpads of the mice. Both imaging studies clearly disclosed SPIO-labeled DCs homing into the draining lymph nodes (**Fig. 2**).

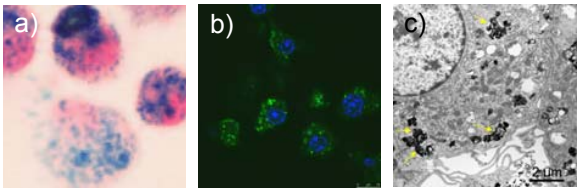

Figure 1. a) Perls' staining showed the blue-stained iron-containing particles within cytoplasm of DCs; b) CLSM investigation of the SPIO-labeled DCs; c) TEM image of DCs labeled with SPIO probes (yellow arrows: SPIO nanocomposites shown as high density particles).

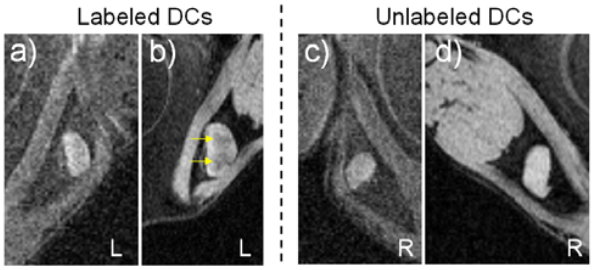

Figure 2. *In vivo* imaging of DC home to the draining lymph node (LN) under a 7T MR scanner. a) and b) were the same left (L) lymph node before and 72h after injection of 1×10^6 cells. Localized decreased intensity was noted within the LN (yellow arrows). Comparatively, the right (R) side lymph node (showed in c and d) did not exhibit the signal intensity decrease.

Conclusion: MRI probes Alkyl-PEI2k/SPIO can be used for high efficiency labeling of DCs without exhibiting cellular toxicity, and migration of labeled cells to the draining lymph node were visible under MRI.

References: 1. Liu G, Ai H, et al. *Biomaterials*, 2011;**32**:528-537. 2. Xie J, Liu G, Ai H, Chen X et al. *Acc Chem Res*. 2011;**44**:883-892. 3. Shan L. *NIH Molecular Imaging and Contrast Agent Database (MICAD)*, http://www.ncbi.nlm.nih.gov/books/NBK52895/. 4. Lutz MB, et al. *J Immunol Methods*. 1999;**223**:77-92.