Intralymphatic cancer cell tracking with two MRI contrast agents: SPIO / quantum dot cell labeling with Gd-dendrimer lymphangiography in the mouse model

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Introduction: *In vivo* visualization of cancer cell migration within the lymphatic system facilitates understanding of lymphatic metastasis, but few imaging methods are capable of providing such imaging. In this study, we dual-labeled cancer cells with two different nano-particles; a small particle of iron oxide (SPIO) and a quantum dot (QD), and tracked these dual-labeled cancer cells in the lymphatic system in mice using *in vivo* MRI and optical imaging. In order to demonstrate the actual lymphatic channels MR lymphangiography was also performed with a gadolinium-dendrimer-based MRI contrast agent (Gd-G6), which shortened T1. Thus it was feasible to visualize the labeled cancer cells within the draining lymphatic basins on a single MR image.

Materials & Methods: A human breast cancer cell line (MDA-MB468) was dual-labeled with both QD (Qtracker655, Invitrogen) and SPIO nanoparticles (Feridex, AMAG Pharmaceutical) with the protamine sulfate method. One million of dual-labeled cancer cells were intracutaneously injected into the paws of mice 24 hrs prior to imaging. MRI was performed with a 3T-clinical MRI scanner (Achieva,Phillips) and a 1-inch saddle type coil. After acquiring non-contrast whole body images of an anesthetized mouse with 3D-T1-FFE sequence (matrix:512x512, TR:16 ms, TE:2.302 ms, FA:30º), 10 μL of Gd-G6 (30 mM) was intracutaneously injected into the ipsilateral paw of the mouse, and MR lymphangiography was acquired with the same 3D-T1-FFE sequence. Following MRI, the same mouse underwent *in vivo* optical imaging using a fluorescence surgical microscope (MVX10, Olympus) with appropriate filter settings. Finally, lymph nodes were resected and imaging findings were histologically validated with H&E staining, Prussian blue staining, and fluorescence.

Results: Cancer cells were successfully labeled with the both QDs and SPIOs. *In vivo* MRI clearly visualized labeled-cancer cells migrating from the paw to the axillary lymph nodes (Fig. 1-A). After injection of Gd-G6, cancer cells and lymphatic basins from the paw were simultaneously depicted with the same MRI sequence (cells dark, lymphatics bright) (Figs. 1-B, C). *In vivo* fluorescence imaging showed tiny cancer cell clusters in the axillary lymph node with high spatial resolution (Fig. 1-D). These labeled cancer cells were also histologically confirmed.

Conclusion: With this method, both the lymphatics and labeled cancer cells within the lymphatics can be simultaneously visualized with a single MRI sequence, while optical imaging enables high-spatial resolution cancer cell tracking in the same mouse can provide a micro-distribution of cancer cells within lymph nodes. These images can provide detailed information of the fate of cancer cells within lymphatic system *in vivo*, and thus, can be a powerful research tool for lymphatic metastases in animal studies.

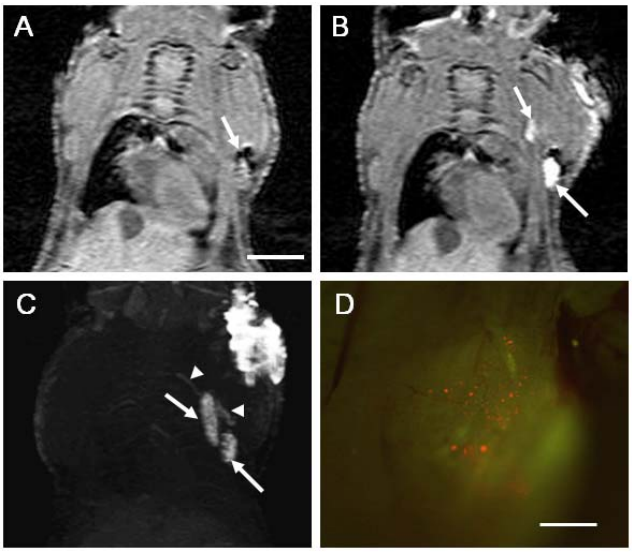

Fig.1 T1-FFE MR image shows iron-labeled cancer cells in the peripheral of the left axillary lymph node (dark areas in A: arrow). After administration of dendrimer-based MR contrast agents into the left paw, the cancer cells and lymphatic basin are simultaneously depicted (lymph nodes: arrows in B and C, lymphatic ducts: arrow heads in C). Fluorescence images shows optically labeled cancer cells (red in D) in an axillary lymph node with high spatial resolution. Note that skins were removed for fluorescence imaging. Scales in MR and fluorescence images are 5 mm and 1 mm, respectively.