

Imaging Lymphatics With A Variety of Near-Infrared-Labeled Optical Agents

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ABSTRACT

The lymphatic system is a specialized network of the circulatory system responsible for fluid balance in the body, absorption and transport of fats and fatty acids, and supporting the immune response. Enhanced permeability and retention (EPR) is a phrase that describes phenomena of the tumor microenvironment, in which vasculature is relatively discontinuous, allowing molecules to diffuse from the bloodstream into the surrounding tumor tissue. In addition, the lymphatic drainage for these EPR regions is poor, further allowing molecules to accumulate. Near-infrared (NIR) imaging technology can be used noninvasively to visualize accumulation of labeled fluorophores within tumor tissue, providing sensitive and high resolution detection of tumors in mice. Imaging of fluorophores such as IRDye[®] 800CW (em: 789 nm) or IRDye 700DX (em: 700 nm) spectrally minimizes interfering autofluorescent signals that generate background noise, which improves image resolution and quality. Images presented here demonstrate the EPR effect in the tumor microenvironment, tracking of lymph flow, and identification of lymph nodes of interest with a variety of NIR-labeled optical agents.

DISCUSSION

Demonstration of the lymphatic drainage in studying neoplasms can be two-fold, early identification of lymph flow and late identification of sentinel lymph nodes and/or the lymph drainage basin (1, 2). We have fluorescently labeled several molecules to evaluate both early and late lymphatic features with the Pearl[®] Imager. Within minutes of an intradermal injection of an agent, flow is noted in lymph ducts in close proximity to the injection site. Once the agent is in the lymph system, it is propelled towards the regional node.

IRDye 800CW-labeled hyaluronan (HA), polyethylene glycol (PEG), or small peptide (PEP) were injected intradermally in athymic *nu/nu* mice and monitored for their ability to highlight lymph tracking. Figure 1 provides images of IRDye 800CW HA and PEP after intradermal injections. This route of administration generally requires less expertise and typically involves a reduced volume of imaging agent. A drape or cover was used to shield the intense signal emitting from injection site which can be detrimental to visualizing the resulting lymph track.

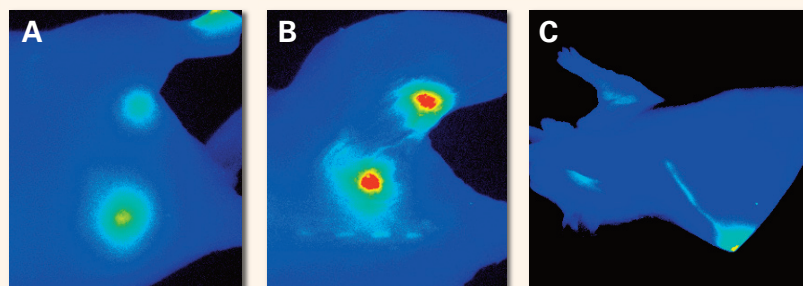


Figure 1. An athymic *nu/nu* mouse was imaged 4 d post-injection of IRDye 800CW HA (0.45 nmol/3 μ L; intradermal). Ischial and popliteal lymph nodes of the right hind leg are visible before (A; 170 μ m) and after (B; 85 μ m) skin was removed for confirmation of fluorescent signal location. Signals are retained for extended periods of time in lymph nodes and vessels, depending on the imaging agent. C) Minutes after IRDye 800CW PEP was administered intradermally at the base of the rib cage in a nude mouse, a lymph track is noted.

Studies have demonstrated the effectiveness of noninvasive fluorescent optical imaging using near-infrared fluorophores to monitor propulsive lymph function in mice (3). Analysis of signal level changes between connected nodes (popliteal and ischial nodes) of the hind limb are presented in Figure 2A and 2B. Propulsion of the lymph agent from the popliteal node to the ischial is detected in images captured 28 sec apart with a reduction in node signal as measured by Pearl Cam software. Subsequently, the ischial node signal increased as it received the pulse from the popliteal node. This is shown graphically in Figure 2C.

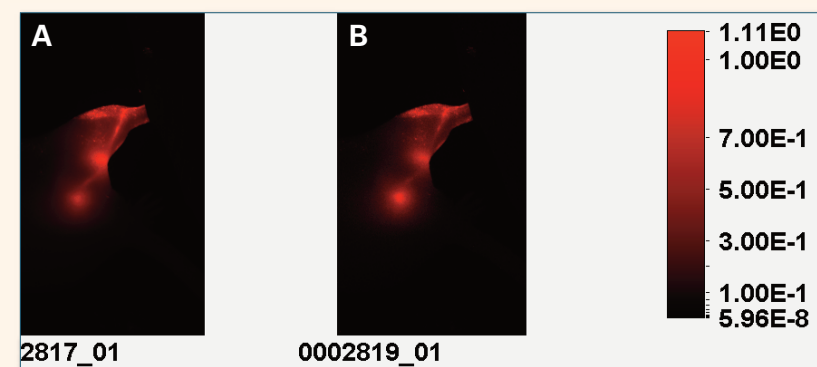
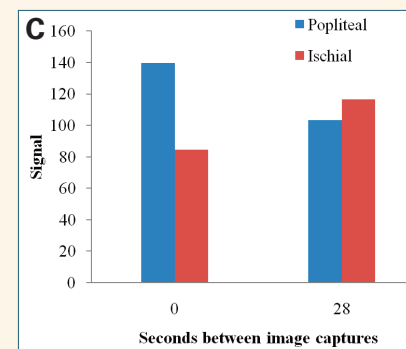


Figure 2. To demonstrate the pulsatile effect of lymph movement, signal intensities from the popliteal and ischial lymph nodes in a mouse were measured after the animal received an intradermal injection of IRDye 700DX HA (0.45 nmol; A and B). C) Graphical representation is shown where, after 28 sec, the signal in the popliteal node is reduced and the signal in the ischial node is increased. The signal reduction from the popliteal node is comparable to the resulting signal increase in the ischial node.



Hyaluronan has several cell surface receptors, namely, CD44, RHAMM (receptor for hyaluronan mediated motility; CD168), LYVE-1 (lymphatic vessel endothelial HA receptor-1), HARE (hyaluronan receptor for endocytosis), layilin, and Toll-4. The agent is catabolized and removed primarily by the liver and lymphatics. Intravenous administration of IRDye 800CW HA allows the agent to localize in lymph nodes where they can be visualized (Figure 3).

Figure 3. Male athymic *nu/nu* mouse received IRDye 800CW HA (hyaluronan) intravenously (1 nmol) and imaged 24 hours post-injection. Para-aortic, gastric, and pancreaticoduodenal lymph nodes (yellow arrows) are visible in this view of the abdominal cavity after removal of the liver, kidneys, and intestines.

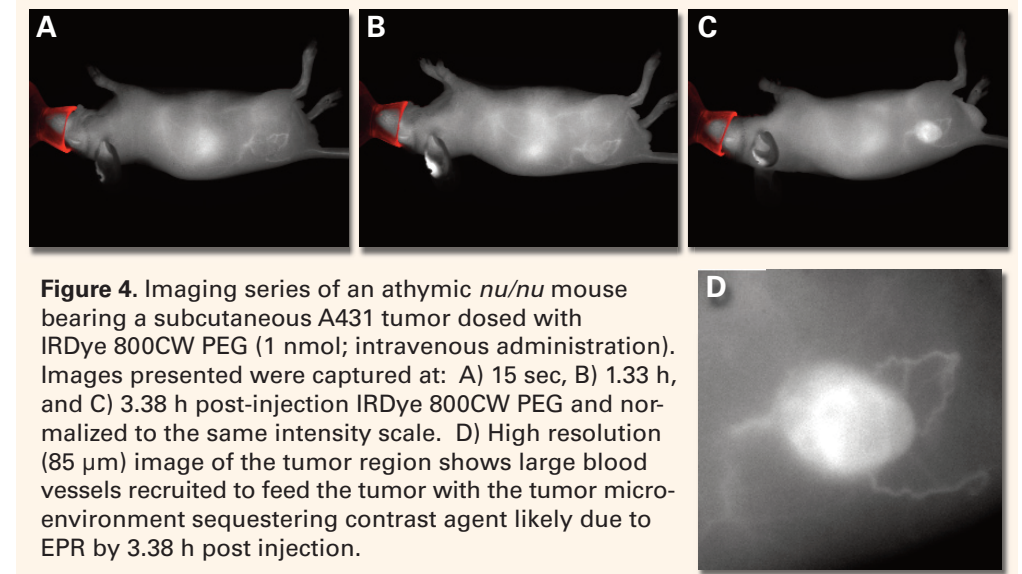
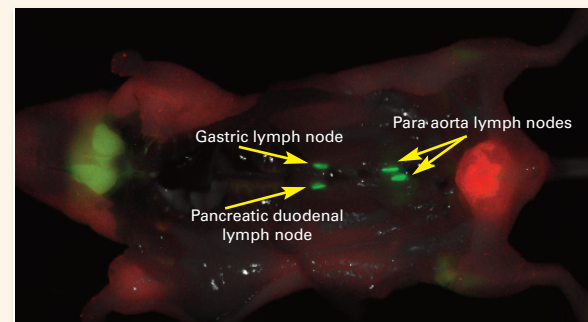


Figure 4. Imaging series of an athymic *nu/nu* mouse bearing a subcutaneous A431 tumor dosed with IRDye 800CW PEG (1 nmol; intravenous administration). Images presented were captured at: A) 15 sec, B) 1.33 h, and C) 3.38 h post-injection IRDye 800CW PEG and normalized to the same intensity scale. D) High resolution (85 μ m) image of the tumor region shows large blood vessels recruited to feed the tumor with the tumor microenvironment sequestering contrast agent likely due to EPR by 3.38 h post injection.

Polyethylene glycol (PEG), a soluble synthetic polymer, has properties suitable for biomedical applications. PEG is commonly used as a carrier agent in drug development to alter the pharmacokinetics of an agent and/or control its biodistribution *in vivo*. [4, 5] IRDye 800CW PEG Contrast Agent is a near-infrared labeled contrast imaging agent designed to exploit the EPR of leaky or discontinuous vascular endothelium. EPR is a feature common to many tumor microenvironments. When given intravenously, the agent appears to have a diffusion coefficient that allows the compound to remain in circulation for an extended period of time (Figure 4).

CONCLUSIONS

We have demonstrated the effective use of near-infrared optical agents for visualizing the lymphatic and circulatory systems in nude mice with the Pearl Imager. The NIR advantage of lower background noise and autofluorescence facilitates the detection of fine features including lymph and blood vessels, and EPR in early tumor microenvironment development.

References

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