

Sentinel node imaging of the breast in mice using a dual-labeled nano-sized MRI/near infrared (NIR) optical hybrid contrast agent, G6-Cy5.5

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Introduction

The presence of lymph node metastases greatly affects prognosis of breast cancer patients. Thus, sentinel lymph node (SLN) biopsy has become routine in the surgical management of breast cancer. Two separate imaging agents are typically used to accurately locate the sentinel node(s) and to minimize invasiveness, a radiolabeled macromolecule such as Tc-99m Albumin and an optical blue dye (isosulfan). We have recently developed a method, MR lymphangiography (MRL), to detect the lymphatic flow and draining lymph nodes using dendrimer-based nano-sized contrast agents¹. Using the G6 (~9 nm in diameter) imaging agent, we were able to clearly detect SLNs of breast cancer and indicate their location through the skin in mice². However, MRL cannot be performed intraoperatively. To directly visualize the SLNs during surgery, we have synthesized a single hybrid probe, which could be used for both MR and near infrared (NIR) optical imaging. This could allow real time guidance to the surgeon during removal of the SLN.

Methods

We synthesized a macromolecular MRI/NIR optical contrast agent based on a gadolinium labeled contrast agent using generation-6 polyamidoamine dendrimers (PAMAM G6) with the addition of Cy5.5 dye. The hybrid contrast agent (G6-Cy5.5) contains ~ 172 Gd ions and 2 Cy5.5 molecules per one molecule of the G6 agent. We examined lymphatic drainage from the left breasts in normal mice at 10, 20, and 30 min post-injection of G6-Cy5.5 by MRL using a 3-Tesla clinical scanner (Signa Excite, GE) equipped with a modified Alderman-Grant resonator mouse coil. The fast-spoiled gradient echo (fSPGR) sequence was used for MRL (TR/TE= 9.2/2.3 msec, flip angle 30°, scan time 3:32', 4 NEX, FOV 8x4 cm, matrix size 512x256). Immediately after the MRI scan, we performed a NIR optical image and an image-guided surgery to confirm the SLN. NIR optical imaging was performed with the Maestro spectroscopic imaging unit (CRI; excitation 615-665 band pass filter; emission 720 nm). We have studied two issues; 1] Dose escalation of G6-Cy5.5 (from 100 - 750 nmol Gd / ~1.2-9.3 nmol Cy5.5) to determine the optimal dose. 2] Correlation between MRI and NIR fluorescence signal of 30 lymph nodes (superficial neck, lateral thoracic, and axillary LNs in 10 consecutive mice) to evaluate the best method for the identification of the "true" SLN.

Results

To consistently identify SLNs on MRL, injection of 25µL of 30mM [750 nmol; Gd basis] G6-Cy5.5 was required (Fig. 1). However, regardless of visualization by MRL, the NIR optical image was able to detect the SLNs with as little as 1 nmol [Cy5.5 basis] during surgery. In contrast, although the NIR optical image failed to identify the SLN external to the body and close to the injection site in the mammary pad (*i.e.* supraclavicle LN, lateral thoracic LN), MRL consistently identified the SLNs if the injected dose was sufficient (Fig. 1). All SLNs could be easily identified and resected under NIR optical imaging-guided surgery (Fig. 2). Although external NIR signal did not correlate with MRI signal, NIR signal from the surgical specimens perfectly correlated with MRI signal intensity in 10/10 mice.

Conclusion

We have successfully synthesized a nano-sized MRI/NIR optical hybrid contrast agent G6-Cy5.5. This agent could be used for preoperative mapping of SLNs as well as for intraoperative guidance. The SLNs could be easily identified using the G6-Cy5.5 agent with MRI and during surgery with NIR optical imaging.

1. Kobayashi, H. et al. *Cancer Res.* 63, 271-6 (2003). 2. Kobayashi, H. et al. *J Natl Cancer Inst* 96, 703-8 (2004).

Fig. 1. SLN imaging with MRI and NIR. Fig. 2. NIR images under an image-guided surgery of SLNs using G6Cy5.5MRI/NIR hybrid contrast agent.

