

## ORIGINAL ARTICLE

# Oral contrast enhances the resolution of in-life NIS reporter gene imaging

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Sodium iodide symporter (NIS) reporter gene imaging is an excellent technology for noninvasive cell fate determination in living animals unless the NIS-transduced cells reside in perigastric organs such as the spleen, liver, diaphragm, omentum, pancreas, perigastric lymph nodes or perigastric tumor deposits. Here we report that orally administered barium sulfate enhances CT definition of the stomach, masks background gamma ray emissions from the stomach and enhances signal detection from radiotracer uptake in NIS-transduced organs.

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## INTRODUCTION

Sodium iodide symporter (NIS) is a plasma membrane glycoprotein that mediates uptake of iodine into the thyroid for the biosynthesis of thyroid hormones.<sup>1</sup> NIS is also used as a reporter gene in small and large animal imaging studies and in humans to monitor the pharmacokinetics and fate of gene, cell and virus therapies.<sup>2</sup> Several readily available SPECT or PET tracers, I-123, I-125, I-124 and Tc-99m pertechnetate, are compatible with NIS imaging. Moreover, NIS-mediated uptake of I-131, a beta emitter, enables targeted radiotherapy of tumors.<sup>3</sup> To protect the thyroid from I-131 damage or eliminate thyroid signals in NIS imaging studies, cytomel (liothyronine sodium) can be given to the animal or patient to inhibit NIS and block thyroid uptake of radioisotopes. However, NIS is also expressed in the salivary glands, lactating mammary glands and gastric mucosa.<sup>4</sup> As I-131 is not retained in these tissues, there is minimal organ damage. However, uptake of NIS radiotracers by the gastric mucosa and release into the gastric lumen results in a strong signal in the stomach, limiting the application of NIS reporter gene imaging in the perigastric region.

Contrast agents are given in CT imaging protocols to aid differentiation of anatomic structures and improve lesion localization.<sup>5</sup> Oral contrast media such as barium sulfate opacifies and delineates the intestines to improve diagnosis in <sup>18</sup>F-FDG PET/CT scans.<sup>6</sup> Typically, contrast agents are diluted to minimize masking of the <sup>18</sup>F-FDG PET signals and the PET images are attenuation corrected to compensate for the radiodense material. We modified this clinically accepted protocol here and, for the first time, show its application to enhance NIS reporter gene imaging studies.

## MATERIALS AND METHODS

### Mice and vectors

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation. Female 5- to 6-week-old athymic mice were purchased from Harlan (Indianapolis, IN, USA), and Rag 1tm 1momj mice were from Jackson (The Jackson

Laboratories, Bar Harbor, Maine, USA). Mice received an intravenous infusion of 25 µg plasmid containing a Sleeping Beauty (SB) transposon encoding a human NIS gene along with 0.5 µg of SB transposase-encoding plasmid (pCMV-SB100x) by hydrodynamic injection (2 ml). Plasmid contains a SB transposon encoding the human NIS gene downstream of a EF1 promoter and the SB100 transposase regulated by a CMV promoter. In another liver gene delivery protocol, athymic mice received 10<sup>9</sup> vp Ad5-CMV-NIS intravenously and were imaged 7 days later. Human ovarian cancer cells SKOV3 were stably transduced with lentiviral vectors encoding human NIS to yield SKOV3-NIS. Athymic mice received 5 × 10<sup>6</sup> SKOV3-NIS cells per 250 µl saline by intraperitoneal injection. Growth of the omental tumors was monitored in the animals over the next 2–3 weeks.

### Imaging studies and image analyses

Animals were fasted overnight by withholding food for 16–18 h. The next day, animals were given an intravenous injection of radiotracer (I-125) at 200–300 µCi as indicated. One hour later, mice were imaged. Immediately before imaging, some animals received 350 µl undiluted barium sulfate (40% w/v, Tagitol V, E-Z-EM, Lake Success, NY, USA) by oral gavage using a 22-gauge plastic feeding tube (Instech Laboratories, Plymouth, PA, USA). Mice were imaged on a dual-modality SPECT-CT scanner (U-SPECT-II/CT, MILabs, Utrecht, The Netherlands).

### SPECT/CT imaging system

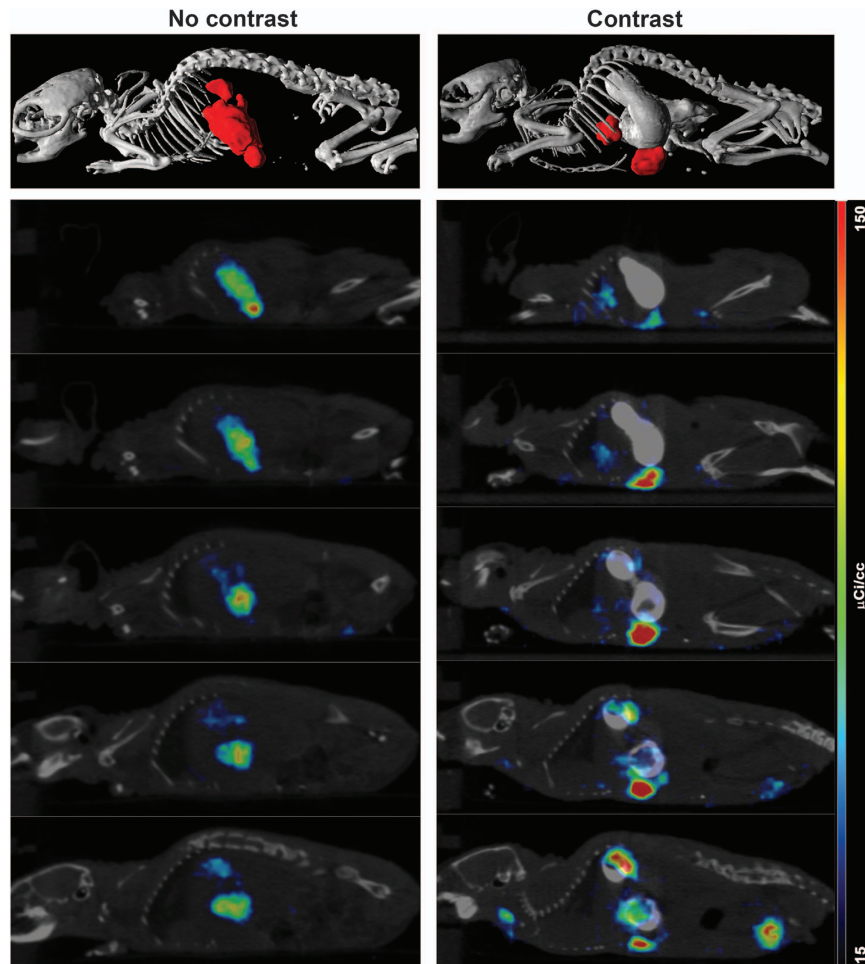
SPECT/CT acquisitions were performed using the U-SPECT-II/CT scanner (MILabs, Utrecht, The Netherlands). This system offers functional and anatomic imaging of small animals, with a SPECT resolution of <0.35 mm using a mouse collimator with 0.35 mm pinholes or <0.8 mm (with a rat/mouse collimator with 1.0 mm pinholes). The SPECT module is described in Van der Have *et al.*<sup>7</sup> The SPECT component consists of a triple headed gamma camera equipped with a 75 pinhole cylindrical collimator (each with an aperture of 1.0 mm). During scanning, mice were fixed in a horizontally positioned plastic bed. Scan volumes for both the SPECT and CT were selected based on orthogonal optical images provided by integrated webcams. Micro-CT image acquisition was performed in 4 min for normal resolution (169-µm square voxels, 640 slices) or 12 min for high resolution (85-µm square voxels, 1280 slices) at 0.5 mA and 60 kV. Image acquisition time was ~20 min for SPECT (69 projections at 50 s per bed position). All pinholes focus on a single volume in the center of the tube, and by using an XYZ stage large volumes up to the entire animal can be

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**Figure 1.** Representative fused SPECT/CT images of mice bearing omental SKOV3-NIS that were fed oral contrast or no contrast. Coronal view of sequential slices shown in eight slices increment (0.17 mm thickness per slice).

scanned at uniform resolution.<sup>8</sup> Coregistration of the SPECT and CT images was performed by applying pre-calibrated spatial transformation to the SPECT images to match with the CT images.

#### Image reconstruction and data processing

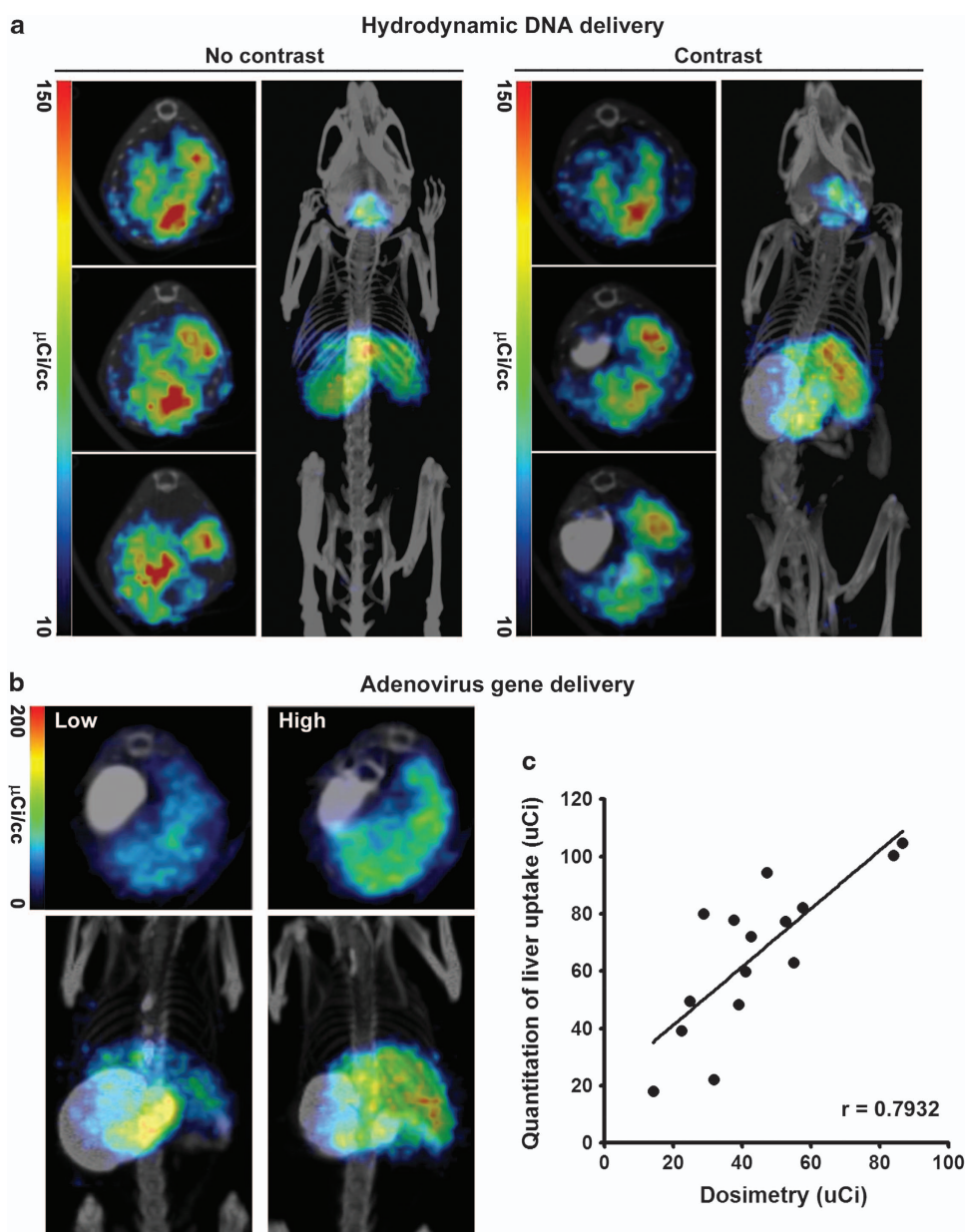
SPECT reconstruction was performed using a pixel-based accelerated iterative ordered subset algorithm based on maximum-likelihood expectation maximization. Images were reconstructed using a precalculated matrix with six iterations using 16 subsets.<sup>9</sup> CT data were reconstructed using a Feldkamp cone beam filtered back-projection algorithm (NRecon v1.6.3, Skyscan, Bruker-MicroCT, Kontich, Belgium). After reconstruction, SPECT images were automatically registered to the CT images according to the pre-calibrated transformation, and re-sampled to the CT voxel size. Co-registered images were further rendered and visualized using the PMOD software (PMOD Technologies, Zurich, Switzerland). A 3D-Gaussian filter (0.8 mm FWHM) was applied to smooth noise, and lookup table scale (LUTs) were adjusted for good visual contrast. Reconstructed images were visualized as both orthogonal slices and maximum intensity projections. Maximal intensity projection videos and three-dimensional rendering of regions of interests were performed on the PMOD software.

## RESULTS AND DISCUSSION

In our studies, undiluted barium sulfate (40% w/v) was used to deliberately block the radiotracer signals in the stomach, and images were processed without attenuation correction. Mice were fasted overnight and fed contrast immediately before anesthesia. We first evaluated whether oral contrast could help delineate

NIS-expressing omental tumors that lie immediately next to the stomach. When injected intraperitoneally into mice, human ovarian cancer cells home to and engraft at the omentum. Mice bearing NIS-expressing SKOV3 tumors (SKOV3-NIS) received one intravenous dose of 300  $\mu$ Ci I-125 and were imaged on a dual-modality SPECT/CT scanner 1 h later. Co-registered SPECT/CT fused images show strong isotope uptake in the perigastric region (Figure 1 and Supplementary Video 1), but it is difficult to differentiate between NIS signals from the stomach and tumor(s) in the absence of oral contrast. Another cohort of mice was given 350  $\mu$ l undiluted barium sulfate by oral gavage immediately before scanning. The stomach is now clearly discernible in the CT images, especially after 3D volume rendering, which clearly shows the u-shaped structure of the CT opaque stomach (Figure 1 and Supplementary Video 1). Analysis of SPECT images indicate isotope uptake of 28.78–99.05  $\mu$ Ci per  $\text{cm}^3$  SKOV3-NIS tumors. In some instances, the isotope signals in the stomach are not totally blocked if the oral contrast does not completely fill the stomach due to presence of food in the animal. This is exemplified in slice 5 of the mouse that received oral contrast (Figure 1). Despite incomplete opacification of the entire gastric pouch, the oral contrast serves to identify the stomach enabling the investigator to confidently differentiate between signals from the stomach and tumor (Supplementary Video 1).

Here, barium sulfate contrast significantly attenuated isotope signals in the stomach lumen, thus enabling reliable detection of nearby NIS-expressing cells. For this method to work effectively,



**Figure 2.** (a) Representative transverse SPECT/CT sections of liver post hydrodynamic DNA gene delivery into Rag 1tm 1momj mice that did not receive (left) or were fed (right) oral contrast immediately before scans. Images showed fusion transverse view of different positions of the stomach, from head to feet of animal. (b) High resolution imaging of relative levels of *NIS* gene expression at day 7 post intravenous adenovirus type 5-NIS deliveries into athymic mice (left). (c) Quantitative analyses. There is a strong correlation between dosimetric measurements and region of interest measurements of the liver from the SPECT images. Pearson's correlation  $r = 0.79$ ,  $P = 0.0004$ . All mice were given I-125 and were imaged 1 h later.

the animals should be fasted for at least 12–16 h before imaging to empty the stomach contents. Twice diluted barium sulfate or smaller volumes were less effective at masking the stomach SPECT signals (data not shown). Oral gavage immediately before scanning works best for our purpose of opacifying the stomach. Imaging at longer times post gavage results in transit of the barium sulfate further down into the intestines and will help identify NIS-positive lesions (if any) in the bowels.

We next assessed the value of oral contrast in imaging NIS expression in two different liver gene delivery protocols. Rag 1tm 1momj mice were given a hydrodynamic (2 ml) intravenous injection of 25  $\mu\text{g}$  plasmid DNA encoding *NIS*. Mice were given 200  $\mu\text{Ci}$  I-125 and imaged 1 h later, with or without oral barium

sulfate (350  $\mu\text{l}$ ). Because of high isotope uptake by the stomach, it is difficult to discern between NIS expression in the liver and stomach in the absence of contrast (Figure 2a). However, if oral contrast was given to opacify the stomach, uniform NIS expression in the liver due to plasmid gene delivery can now be reliably detected (Figure 2, Supplementary Video 2).

We also evaluated whether detection sensitivity of NIS expression *post adenovirus* gene delivery could be enhanced by oral barium sulfate (Figure 2b). Athymic mice were given one intravenous dose of type 5 adenovirus expressing NIS at  $10^9$  vector particles per animal and imaged 7 days later. As shown in the transverse slices and maximal projection images (Figure 2b, Supplementary Video 3), uniform gene expression in the liver is

readily discernible in the mouse that received barium sulfate. High resolution imaging of *NIS* gene expression in the liver is now feasible and it is easy to discern between low and high levels of gene expression using this noninvasive method of the reporter gene imaging (Figure 2b). Livers of animals were harvested and the amount of radioactive uptake due to *NIS* expression was measured using a dose calibrator. Because of masking of gamma signals from the stomach, it is also possible to accurately quantitate *NIS* expression in the liver from regions of interest measurements using image analysis softwares. There is a strong positive correlation between the actual counts and quantitation by regions of interest measurements from SPECT image analysis (Pearson's correlation  $r = 0.79$ ,  $P = 0.0004$ , Figure 2c).

In summary, we demonstrated the value of oral contrast barium sulfate in facilitating the detection of *NIS*-expressing cells for whole-animal imaging studies. The oral contrast protocol is modified to not only identify the stomach and delineate it but also the radiodense material to serve as a block to mask the stomach signal from endogenous *NIS*-mediated isotope uptake, thereby increasing the sensitivity of *NIS* detection.

### CONFLICT OF INTEREST

SJR and KWP are co-founders of Imanis Life Sciences, a reporter gene imaging company. SJR, KWP and Mayo Clinic own equity in Imanis.

Supplementary Information accompanies the paper on Cancer Gene Therapy website (<http://www.nature.com/cgt>)

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