

A “New” Reporter in the Field of Imaging Reporter Genes: Correlating Gene Expression and Function of the Sodium/Iodide Symporter

By 1995, more than 150 human gene therapy clinical trials were in progress in the United States alone (1). More than 10 y later, gene delivery in humans remains a considerable challenge for numerous potential applications, because it is often limited by poor delivery specificity, inadequate transfer efficiency, and lack of correlation between expression and function. The limitations of clinical gene therapy led to the emergence of an exciting new opportunity for noninvasive imaging in living subjects: detection and monitoring of molecular biologic events.

As the initial shortcomings of gene therapy became apparent, the challenge of imaging processes related to

See page 182

gene transfer, expression, and function was seized by several investigators in the fields of nuclear and molecular imaging. Molecular biologists had long used reporter assays to detect gene expression *in situ*, including the use of the bacterial genes chloramphenicol acetyl transferase and β -galactosidase. With the development of reporter probes, the reporter gene paradigm was adapted to *in vivo* molecular imaging, and pioneering efforts developed along 3 fronts:

nuclear imaging (PET, SPECT, γ -scintigraphy, and autoradiography) (2,3), MRI (4,5), and optical imaging (6,7). The idea of the reporter gene as an imaging gene had become a reality.

In the last 3 y, one of the “new” reporters to emerge in the field of imaging reporter genes, the sodium/iodide symporter (NIS), is ironically one of the oldest examples of a molecular imaging and therapy target in nuclear medicine. Selective localization of $^{131}\text{I}^-$ began to be exploited in the 1940s and 1950s for selection and treatment of thyroid patients deemed to be poor surgical candidates (8). It later became well established that uptake by the NIS, and subsequent metabolic trapping by organification in the form of hormones, was the mechanism that allowed thyroid imaging and therapy. In recent years, the same principles of metabolic trapping and high signal amplification have become a common paradigm around which imaging reporter genes and probes have been designed.

The elegant biochemistry that made the NIS one of the first nuclear medicine targets also makes it an attractive candidate for transgene imaging and therapy applications. Preclinical studies published in the last 3 y have investigated the potential of the NIS to function in both capacities in non-thyroid cancer models. After *ex vivo* retroviral transduction, Jhiang’s group imaged human NIS gene expression in intracerebral F98 rat glioma allografts, using $^{99\text{m}}\text{TcO}_4^-$ and $^{123}\text{I}^-$ scintigraphy (9), and in pulmonary tumors in mice, correlating uptake and perfusion defects with $^{125}\text{I}^-$ and $^{99\text{m}}\text{Tc}$ -macroaggregated albumin SPECT (10). Jhiang’s group also showed that treat-

ment with $^{131}\text{I}^-$, $^{125}\text{I}^-/^{131}\text{I}^-$, or $^{188}\text{ReO}_4^-$ significantly prolonged the survival of F98/NIS-bearing rats (9,11), demonstrating that exogenous NIS expression can be exploited for therapy of nonthyroid tumors. Shin et al. (12) compared the imaging reporter characteristics of NIS with the more conventional herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene in mice bearing transfected CT-26 colon carcinoma xenografts. Biodistributions of $^{125}\text{I}^-$ and ^{125}I -iodovinyldeoxyuridine targeting NIS and HSV1-tk, respectively, showed that tumor uptakes per unit transgene expression were comparable for the two, and established NIS-expressing tumors were clearly detected by $^{131}\text{I}^-$ scintigraphy.

Also during the last 3 y, the NIS has been evaluated as a reporter for imaging gene therapy in rodent models. Groot-Wassink et al. performed high-resolution $^{124}\text{I}^-$ PET studies of adenoviral delivery of the human NIS gene in nude mice (13,14). Systemic administration of NIS recombinant adenovirus allowed transgene imaging in the liver, spleen, lungs, pancreas, and adrenal glands, whereas tumor xenografts could be detected after intratumoral injection of the viral vector. Adenoviral transduction of human NIS has also been used to study cardiac and pulmonary gene transfer in rat models. Lee et al. (15) used dual-radionuclide scintigraphy with $^{123}\text{I}^-$ and $^{201}\text{Tl}^+$ to demonstrate that myocardial NIS expression persisted in rats 4 d after local delivery of recombinant adenovirus. Washout of ^{123}I from the myocardium was relatively slow, approximately 3.3% per hour. After introducing an adenoviral

Received Sep. 17, 2005; revision accepted Sep. 22, 2005.

For correspondence or reprints contact: Michael R. Lewis, PhD, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, 379 E. Campus Dr., University of Missouri-Columbia, Columbia, MO 65211.

E-mail: LewisMic@missouri.edu

vector into the lungs of rats, Niu et al. (16) were able to image NIS expression by $^{99m}\text{TcO}_4^-$ γ -scintigraphy and $^{124}\text{I}^-$ small-animal PET until 17 d after administration.

These proof-of-principle studies suggest that, even in the absence of organification, the NIS has considerable potential for transgene imaging and therapy. To this end, several laboratory methods have been used to correlate NIS gene expression and molecular imaging. Shin et al. (12) found a direct linear relationship between in vitro radioiodide uptake (RAIU) and NIS messenger RNA levels for transfected CT-26 mouse colon cancer cells. Both Lee et al. (15) and Niu et al. (16) used dual-gene adenoviral vectors expressing NIS and enhanced green fluorescent protein (EGFP) to evaluate in vivo transduction. Microautoradiography, fluorescence microscopy, and NIS immunohistochemical staining colocalized in the myocardia of rats after local injection of a dual-expressing NIS/EGFP adenoviral vector (15). Similarly, $^{99m}\text{TcO}_4^-$ uptake and microscopic fluorescence were clearly detected in the lungs of rats in which dual NIS/EGFP recombinant adenovirus had been distilled via the nostrils (16). After systemic administration of an NIS single-gene vector, Groot-Wassink et al. correlated ex vivo counting of liver biopsy specimens (14), messenger RNA concentrations (13,14), and immunohistochemical staining (14) with $^{124}\text{I}^-$ imaging in an adenoviral dose-dependent manner. These studies demonstrated that PET can provide quantitative information on NIS gene expression in living animals.

However, induction of exogenous NIS expression and detection of its function can sometimes produce confounding results. For example, Niu et al. (16) did not observe focal uptake of $^{99m}\text{TcO}_4^-$ in the lungs of NIS adenovirus-infected rats at 20 d after administration, even though NIS messenger RNA was easily detected by reverse transcriptase polymerase chain reaction. Reporter gene expression, as measured by an indirect laboratory correlate, may not always

translate into functional imaging, and the relationship between the two clearly warrants further investigation. NIS gene transfer can facilitate RAIU and radiometal mimics, but the extent of this activity can vary widely between different cell types, animal models, and routes of administration. The mechanisms responsible for these differences in NIS expression and function have not been studied previously.

In this issue of *The Journal of Nuclear Medicine*, Vadysirisack et al. (17) have taken the study of NIS reporter gene expression and function a step further, by investigating how posttranslational processing and cell trafficking of exogenous NIS affect RAIU. The authors established FTC133 human thyroid cancer, HeLa human cervical carcinoma, and PC12 rat pheochromocytoma cells in which the NIS transgene was linked to a tetracycline-responsive transactivator. Exposure of these cells to doxycycline resulted in profound up-regulation of NIS, with concomitant increases in RAIU. However, efflux of ^{125}I from all 3 cell lines was extremely rapid, decreasing by 50% in 4 min and to values ranging from <30% to <10% of the initial uptake by 10 min. It was notable that the thyroid cancer cells showed similar behavior to nonthyroid tumor cells. Although metabolic trapping might be expected, the thyroid cell line used, FTC133, is deficient in thyroid peroxidase, a key enzyme that facilitates iodide organification. Comparison of FTC133 RAIU and efflux to a matched cell line lacking NIS but having functional thyroid peroxidase, as well as to thyroid carcinoma cells expressing both NIS and thyroid peroxidase, would provide useful information on the relative performance of exogenous versus endogenous NIS for monitoring gene activity.

Rapid efflux of ^{125}I notwithstanding, it would also be interesting to know how NIS would report from one of these cell lines in vivo. PC12 rat pheochromocytoma cells are tumorigenic, affording the opportunity to

evaluate RAIU and efflux in established NIS-transduced PC12 allografts. Even when in vitro washout is relatively rapid (13), high uptake and significant retention of radioiodide and $^{99m}\text{TcO}_4^-$ has been observed in animals bearing NIS-transduced nonthyroid tumors (12,13). Furthermore, $^{99m}\text{TcO}_4^-$ is used for thyroid imaging in veterinary medicine and for diagnosing incidental clinical findings. These applications suggest that lack of organification and trapping in vivo will not necessarily limit the extension of NIS transgene targeting to clinical imaging and therapy of nonthyroid diseases. However, future development of strategies aimed at enhancing in vivo radioiodide trapping in NIS-transduced cells could improve both signal amplification and treatment response, making this reporter system more robust and versatile for combination gene/radionuclide imaging and therapy.

A significant advance made by Vadysirisack et al. (17) in the understanding of NIS reporter gene expression and function was the correlation of total protein expression with cell-surface trafficking. The concentration of functional NIS protein at the cell surface, rather than the total expression level, determines the degree of NIS-mediated RAIU activity. This question—that is, what posttranslational processes are critical for success?—is not often addressed in the development of imaging reporter genes. Vadysirisack et al. used a cell-surface biotinylation approach, followed by avidin bead capture and Western blotting, to distinguish cell-surface NIS levels from total protein expression quantitatively. As might be expected, cell-surface trafficking of NIS in the 3 cell lines studied was directly proportional to total synthesis. However, the forms of cell-surface NIS versus total NIS differed with cell type. Total NIS in transduced FTC133, HeLa, and PC12 cells was observed predominantly as the fully glycosylated, 90-kDa monomer, with markedly lower concentrations of a 181-kDa dimer and a 65-kDa partially glycosy-

lated species. At the cell surface, the rat PC12 cell line expressed a higher abundance of the dimer, whereas the 2 human cell lines expressed higher amounts of the fully glycosylated monomer. In all 3 cell lines, the immature, partially glycosylated monomer appeared to be confined largely to intracellular compartments. The form of NIS at the surface may have functional implications for RAIU in transduced cells. Interestingly, the ratio of cell surface monomer to dimer expression appears to follow the trend FTC133 > HeLa > PC12, directly proportional to the observed RAIU activities of these cell lines. If, indeed, the monomeric species mediates RAIU more efficiently than the dimer, then additional studies to correlate the form of cell-surface protein expression with activity could prove useful in optimizing NIS reporter gene performance.

Regardless of the biochemical form of NIS expressed at the cell surface, a finding of potential clinical importance was that moderate increases in NIS protein expression resulted in profound increases in RAIU in the cell lines studied. Moreover, increasing cell-surface NIS expression beyond certain levels did not increase RAIU activity further. Taken together, these results highlight both potential advantages and limitations of NIS reporter gene imaging and therapy. Low-level cell-surface expression of NIS protein may afford sufficient signal amplification and therapeutic index for efficient transgene/radionuclide imaging and treatment modalities. Conversely, the ability to correlate diagnostic or therapeutic gene expression levels with radioiodide concentration may be confined to a relatively narrow quantitative range.

For clinical applications, the NIS may have several distinct advantages

over foreign reporter genes such as HSV1-tk and luciferase: The NIS gene product is innate and nonimmunogenic; tissue-specific expression of endogenous NIS is limited; the NIS mediates selective uptake of chemically simple and readily available radiopharmaceuticals; and NIS-mediated radiopharmaceutical kinetics display rapid target tissue uptake and background clearance *in vivo*. The work of Vadysirisack et al. (17) represents an important step forward in defining optimal NIS reporter gene applications. At the same time, this report raises in a new light several highly relevant questions about one of nuclear medicine's oldest "players": What other cellular factors, possibly including the biochemical form of cell-surface NIS, modulate symporter-mediated RAIU? What is the optimal NIS cell-surface expression level for radioiodide targeting, and how does it vary by tissue type? Can we exploit these factors to maximize RAIU and realize the full potential of the NIS as a reporter? The answers may soon provide the keys to practical and effective use of NIS-mediated gene/radionuclide imaging and therapy in the clinic.

Michael R. Lewis, PhD

*University of Missouri–Columbia
Columbia, Missouri*

REFERENCES

1. Cai Q, Rubin JT, Lotze MT. Genetically marking human cells: results of the first clinical gene transfer studies. *Cancer Gene Ther.* 1995;2:125–136.
2. Gambhir SS, Barrio JR, Phelps ME, et al. Imaging adenoviral-directed reporter gene expression in living animals with positron emission tomography. *Proc Natl Acad Sci U S A.* 1999;96:2333–2338.
3. Tjuvajev JG, Avril N, Oku T, et al. Imaging herpes virus thymidine kinase gene transfer and expression by positron emission tomography. *Cancer Res.* 1998;58:4333–4341.

4. Louie AY, Huber MM, Ahrens ET, et al. *In vivo* visualization of gene expression using magnetic resonance imaging. *Nat Biotechnol.* 2000;18:321–325.
5. Weissleder R, Simonova M, Bogdanova A, et al. MR imaging and scintigraphy of gene expression through melanin induction. *Radiology.* 1997;204:425–429.
6. Bennett J, Duan D, Engelhardt JF, et al. Real-time, noninvasive *in vivo* assessment of adeno-associated virus-mediated retinal transduction. *Invest Ophthalmol Vis Sci.* 1997;38:2857–2863.
7. Rehemtulla A, Stegman LD, Cardozo SJ, et al. Rapid and quantitative assessment of cancer treatment response using *in vivo* bioluminescence imaging. *Neoplasia.* 2000;2:491–495.
8. Perlmutter M, Slater S. Which nodular goiters should be removed? *N Engl J Med.* 1956;255:61–71.
9. Cho J-Y, Shen DHY, Yang W, et al. *In vivo* imaging and radioiodine therapy following sodium iodide symporter gene transfer in animal model of intracerebral gliomas. *Gene Ther.* 2002;9:1139–1145.
10. Marsee DK, Shen DHY, MacDonald LR, et al. Imaging of metastatic pulmonary tumors following NIS gene transfer using single photon emission computed tomography. *Cancer Gene Ther.* 2004;11:121–127.
11. Shen DHY, Marsee DK, Schaap J, et al. Effects of dose, intervention time, and radionuclide on sodium iodide symporter (NIS)-targeted radionuclide therapy. *Gene Ther.* 2004;11:161–169.
12. Shin JH, Chung J-K, Kang JH, et al. Feasibility of sodium/iodide symporter gene as a new imaging reporter gene: comparison with HSV1-tk. *Eur J Nucl Med Mol Imaging.* 2004;31:425–432.
13. Groot-Wassink T, Aboagye EO, Glaser M, et al. Adenovirus biodistribution and noninvasive imaging of gene expression *in vivo* by positron emission tomography using human sodium/iodide symporter as reporter gene. *Hum Gene Ther.* 2002;13:1723–1735.
14. Groot-Wassink T, Aboagye EO, Wang Y, et al. Quantitative imaging of Na/I symporter transgene expression using positron emission tomography in the living animal. *Mol Ther.* 2004;9:436–442.
15. Lee K-H, Kim H-K, Paik J-Y, et al. Accuracy of myocardial sodium/iodide symporter gene expression imaging with radioiodine: evaluation with a dual-gene adenovirus vector. *J Nucl Med.* 2005;46:652–657.
16. Niu G, Krager KJ, Graham MM, et al. Noninvasive radiological imaging of pulmonary gene transfer and expression using the human sodium iodide symporter. *Eur J Nucl Med Mol Imaging.* 2005;32:534–540.
17. Vadysirisack DD, Shen DH, Jhiang SM. Correlation of Na⁺/I⁻ symporter expression and activity: implications for use of Na⁺/I⁻ symporter as an imaging reporter gene. *J Nucl Med.* 2006;47:182–190.