Development of a Sodium/Iodide Symporter (NIS)-Transgenic Mouse for Imaging of Cardiomyocyte-Specific Reporter Gene Expression

Joo Hyun Kang, PhD[1,2]; Dong Soo Lee, MD[1]; Jin Chul Paeng, MD[1]; Jae Sung Lee, PhD[1]; Yun Hui Kim, BS[1,2]; Yong Jin Lee, MS[1]; Do Won Hwang, BS[1,2]; Jae Min Jeong, PhD[1,2]; Sang Moo Lim, MD[3]; June-Key Chung, MD[1,2]; and Myung Chul Lee, MD[1]

[1]Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea; and [3]Korean Institute of Radiological and Medical Sciences, Seoul, Korea

Development of a small animal imaging system for differentiated cell-specific reporter gene expression will enable us to image cellular differentiation in vivo. In this study, we developed a sodium/iodide symporter (NIS)-transgenic mouse in which NIS is constitutively expressed as an imaging reporter gene only in cardiomyocytes. **Methods:** To express NIS gene in cardiomyocytes, α-myosin heavy chain (α-MHC)-NIS was constructed and used for the production of NIS-transgenic mice. Twelve lines of positive founder were obtained. The adequacy of the transgenic mouse model was tested by in vivo scintigraphy, microPET, and a biodistribution study. **Results:** The myocardium of transgenic mice showed rapid and intense uptake of ^{131}I, which was much higher than that of the thyroid, and also showed long retention by γ-camera pinhole imaging. The relative uptake ratio of the heart of transgenic mice was 4.6 ± 1.5, which was 3.8 ± 1.2 times higher than that of control wild-type mice. The uptake of the heart was completely blocked by oral administration of $KClO_4$, an NIS inhibitor. The heart of transgenic mouse was also clearly and intensely visualized on microPET using ^{124}I. Biodistribution data of these mice showed the uptake of 40–160 %ID/g (percentage injected dose per gram of tissue) of ^{99m}Tc-pertechnetate in the heart compared with 40–60 %ID/g in the stomach, respectively. NIS expression in the myocardium was confirmed by immunohistochemistry using a NIS-specific antibody. **Conclusion:** We developed a transgenic mouse model to image cardiomyocytes with a γ-camera and microPET using an α-MHC promoter and NIS. The transgenic mouse can be used as an imaging model for cardiomyocyte-specific reporter gene expression and cellular differentiation into cardiomyocytes after cardiac stem or progenitor cell transplantation.

Key Words: sodium/iodide symporter; transgenic mouse; reporter gene; nuclear imaging

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For correspondence or reprints contact: Dong Soo Lee, MD, Department of Nuclear Medicine, Seoul National University College of Medicine, 28 Yeongeon-Dong, Jongno-Gu, Seoul, 110-744, Korea.

E-mail address: dsl@plaza.snu.ac.kr

Molecular imaging is broadly defined as characterization and measurement of biologic processes at the cellular and molecular levels by imaging methods. We can image diverse cellular processes, such as gene expression (*1,2*), protein–protein interaction (*3*), and signal transduction. We also can monitor cancer cells (*4*), immune cells (*5*), and stem cells by introduction of reporter genes using molecular imaging (*6,7*). Sodium/iodide symporter (NIS) expressed in thyroid cells is an intrinsic membrane protein with 13 transmembrane domains. NIS is proposed as an imaging reporter gene because it concentrates radionuclides such as ^{124}I, ^{131}I, and ^{99m}Tc-pertechnetate, which is clearly visualized by conventional γ-camera or PET (*8,9*). Application of genetically modified animals such as transgenic mice to molecular imaging may provide an opportunity to examine cellular differentiation and transdifferentiation after transplantation (*7*). Transgenic mice expressing a reporter gene placed under the control of cardiomyocyte-specific promoter will be a valuable tool for imaging cardiomyocyte-specific gene expression (*10*).

To enable the imaging of cardiomyocyte-specific reporter gene expression, we developed NIS-transgenic mice with cardiomyocyte-specific expression of a constitutively active NIS driven by the α-myosin heavy chain (α-MHC) promoter in cardiomyocytes. The cardiomyocytes of transgenic mice showed excellent characteristics of sustained uptake of radioisotopes on γ-camera and microPET.

MATERIALS AND METHODS

Plasmid Construction

The transgene vector consisted of human NIS (hNIS) coding sequence flanked by full-length α-MHC promoter and a bovine growth hormone (BGH) 3′-polyadenylation sequence. The α-MHC promoter was a gift from Dr. Chang Kyu Oh Park (Macrogen Co.) and a 5.5-kilobase (kb) promoter sequence was excised by *Bam*HI and *Sal* I. The 5.5-kb promoter sequence was subcloned into

pBluescriptII KS (Stratagene) (pKS-MHC). The polymerase chain reaction (PCR) product of hNIS, including the 3′ BGH polyadenylation sequence from FL*-hNIS/pcDNA3 (kindly provided by Dr. Sissy Jhiang, Ohio State University, Columbus, OH) with 5′ *Sal* I and 3′ *Kpn* I restriction sites, was inserted into *Sal* I- and *Kpn* I-digested pKS-MHC. This transgene was named pαMHC-hNIS.

Transfection and In Vitro Radioiodine Uptake Assay

The human hepatoma cell line SK-Hep1, from Korean Cell Line Bank, and rat myoblast cell lines L6 and H9c2, obtained from Dr. Eun-Seok Jeon, were routinely grown in RPMI 1640 medium supplemented with 10% fetal bovine serum. To verify cardiomyocyte-specific expression of the constructed plasmid, pαMHC-hNIS was transfected into the above cells using Lipofectamine plus reagent (Invitrogen) according to the manufacturer's instructions. As a positive control, another human NIS gene flanked with cytomegalovirus promoter (pcDNA-hNIS) was also transfected into the same cell lines.

Radioiodine uptake by cells in vitro was measured as described by Nakamoto et al. (*11*). Briefly, the cells were plated in 24-well plates and cultured until the cells reached confluence ($\sim 1.0 \times 10^6$ cells). The cells were incubated at 37°C for 30 min in 500 μL of Hanks' balanced salt solution (HBSS) containing 0.5% bovine serum albumin, 18.5 kBq (0.5 μCi) carrier-free $Na^{125}I$, and 10 μmol/L of NaI to yield a specific activity of 740 MBq/mmol. The cells were quickly washed with 2 mL of cold HBSS and the radioactivity of detached cells was counted with a γ-counter (Canberra Industries).

Transgenic Mouse Production

The transgene sequence was obtained by excision with *Bss*HII of pαMHC-hNIS for removing the irrelevant pBluescript vector sequence. The purified fragment was microinjected into fertilized oocytes of FVB mice according to standard protocols. Founder animals were produced and mated with wild FVB strain mice. Transgenic offspring were identified by PCR typing of unique transgene sequences from tail genomic DNA using oligonucleotide primers (MHC-NIS1-A, gtggctctctcagtcaacg; MHC-NIS1-AS, acaggggtccaagagcc).

In Vivo Imaging

At the time of imaging, animals were >8 wk old. Animals were anesthetized by an intraperitoneal injection of 40 μL of ketamine and xylazine (4:1) solution. Immediately after injecting 7.4 MBq of ^{131}I into the lateral tail veins, animals were placed in a spread prone position. Dynamic scans (2 min per frame) for 1 h was performed with a γ-camera (ON410; Ohio Nuclear) equipped with a pinhole collimator. To modulate the radioiodine uptake, $KClO_4$, an inhibitor of NIS activity, was administrated orally before radioiodine administration and images were acquired again with the same protocol.

The phenotypical expression of pαMHC-hNIS was quantitatively evaluated on ^{99m}Tc-pertechnetate scans. Eleven mice, which were positive on PCR typing, were sampled and static images were acquired for 5 min, 30 min after intravenous injection of 7.4 MBq of ^{99m}Tc-pertechnetate. As a control group, 4 wild-type mice were scanned with the same protocol. On the acquired images, regions of interest (ROIs) were drawn around the heart and whole body, and the uptake was counted in each ROI. The relative uptake ratio of heart (RUR_{heart}) was calculated and expressed as the ratio of mean heart uptake (counts/pixel) versus the mean whole-body uptake (counts/pixel).

microPET was performed to assess the feasibility of the transgenic mouse for a PET model. Each anesthetized mouse was placed in the prone position on the scanner gantry and injected with 11.1 MBq of ^{124}I into the tail vein. Sixty-minute list mode PET scans were acquired immediately after the radiotracer injection. PET data were acquired using a small-animal dedicated PET scanner (microPET R4; Concorde Microsystems Inc.). Transaxial images were reconstructed from the 20- to 60-min PET data using Fourier rebinning and 2-dimensional order-subsets expectation maximization reconstruction methods (16 subsets and 4 iterations) without correction for attenuation and scatter as $128 \times 128 \times 63$ matrices of size $0.85 \times 0.85 \times 1.21$ mm.

Biodistribution, Histology, and Immunohistochemistry

After imaging, animals were sacrificed and the explanted organs were counted for radioactivity in a γ-counter. Results were expressed as a percentage of the injected dose (%ID) per gram of tissue normalized with respect to a 20-g mouse.

Explanted hearts were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4-μm thickness. The sections were stained with hematoxylin–eosin or with Masson's trichrome for collagen and immunohistochemistry. Sections were incubated with primary antibody for rat NIS (kindly provided by Dr. Shinji Kosugi, Kyoto University), followed by a secondary antibody. Visualization was accomplished using an avidin–biotin–peroxidase complex as described by the manufacturer (Dako).

RESULTS

In Vitro Radioiodine Uptake Assay and Transgenic Mouse Production

The radioiodine uptakes of pαMHC-hNIS transfected myoblast cell lines (L6 and H9c2) were 20 times and 10 times higher than those of parental cells, respectively. However, the radioiodine uptake of a hepatoma cell line (SK-Hep1) transfected with pαMHC-hNIS was the same as that of the control (Fig. 1). These results suggest that α-MHC promoter was active only in cardiomyocytes.

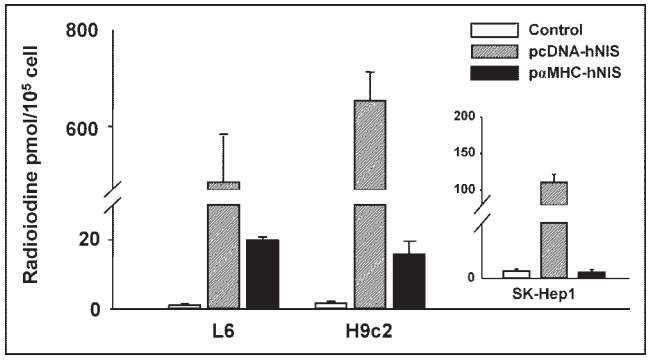

FIGURE 1. In vitro radioiodine uptake by parental cells (white bar), pcDNA-hNIS transfected cells (hatched bar), and pαMHC-hNIS transfected cells (black bar). All data are expressed as means (pmol/10^6 cells) of tetraplicate wells. (Inset) The same transgene was transfected to a hepatoma cell line (SK-Hep1) and pαMHC-hNIS was not expressed in these cells.

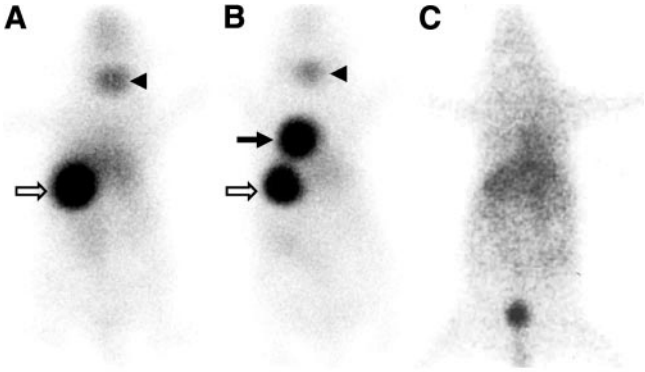

FIGURE 2. Whole-body ^{99m}Tc-pertechnetate scans of control wild-type mouse (A), transgenic mouse (B), and $KClO_4$ challenged transgenic mouse (C). Transgenic mouse shows higher uptake of ^{99m}Tc-pertechnetate in heart (black arrow) than in thyroid (arrowhead) or stomach (outlined arrow). However, uptake was completely blocked by $KClO_4$, an inhibitor for NIS.

In the production of transgenic mice, 12 transgenic founder mice were generated and successfully mated. Seven founders were able to transmit their transgene in Mendelian fashion but 5 founders did not. All of these transgenic animals were phenotypically normal.

In Vivo Imaging

In the scans of transgenic animals, radioiodine uptake in the myocardium of transgenic mice was marked and higher than that of the thyroid or stomach, which are physiologic NIS-expressing organs. The $KClO_4$, a specific NIS inhibitor, completely blocked radioiodine uptake of the heart (Fig. 2). In the quantitative evaluation of myocardial uptake, the RUR_{heart} in transgenic mice was 4.6 ± 1.5, whereas it was 1.2 ± 0.1 in control mice. The RUR_{heart} of the transgenic mice was 3.8 ± 1.2 times higher than that of the control mice. On a dynamic study, radioiodine uptake of the heart increased continuously for 60 min, while the uptake of stomach or background reached anearly plateau, demonstrating long-term retention of radioiodine (Fig. 3).

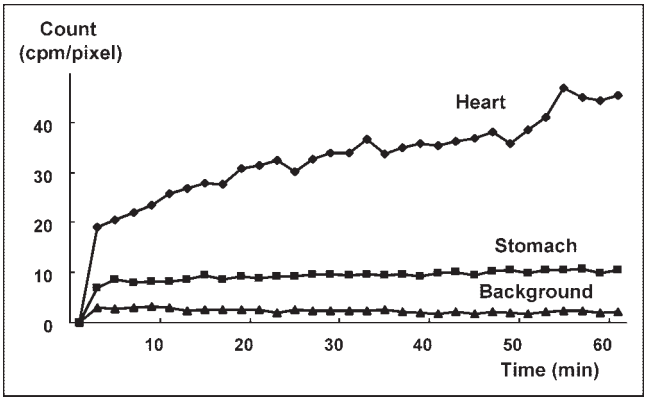

FIGURE 3. Time–activity curves of ROIs on ^{131}I dynamic scan of transgenic mouse. Uptake of heart shows gradual increase during scan time, while uptake of stomach and background reached an early plateau.

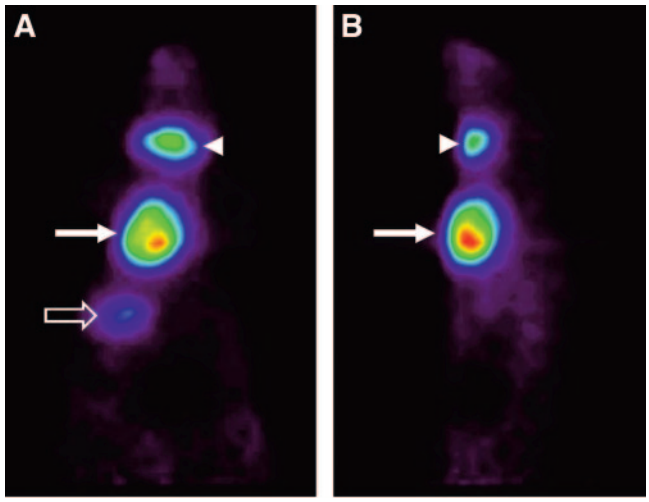

FIGURE 4. microPET image of transgenic mouse using ^{124}I. (A) Coronal section. (B) Sagittal section. Transgenic mouse shows higher uptake of ^{124}I in heart (white arrow) than in thyroid (arrowhead) or stomach (outlined arrow). Transgenic mouse model was also feasible for PET using positron-emitting radioisotope such as ^{124}I.

microPET using ^{124}I also showed a high uptake of radioiodine in the heart of transgenic mouse and the uptake complied well with the findings on γ-camera images (Fig. 4).

Biodistribution, Histology, and Immunohistochemistry

The biodistribution of ^{99m}Tc-pertechnetate in pαMHC-hNIS-transgenic mice was studied in 2 mice that showed the highest and lowest RUR_{heart} (7.2 and 2.5, respectively; Fig. 5). The heart of transgenic mice took up 40 and 160 %ID/g of radioiodine but the stomach accumulated 60 and 40 %ID/g after administration of ^{99m}Tc-pertechnetate. These biodistribution data were consistent with the in vivo images (Fig. 2).

NIS expression in the myocardium was confirmed by immunohistochemistry (Fig. 6). The most of expressed NIS

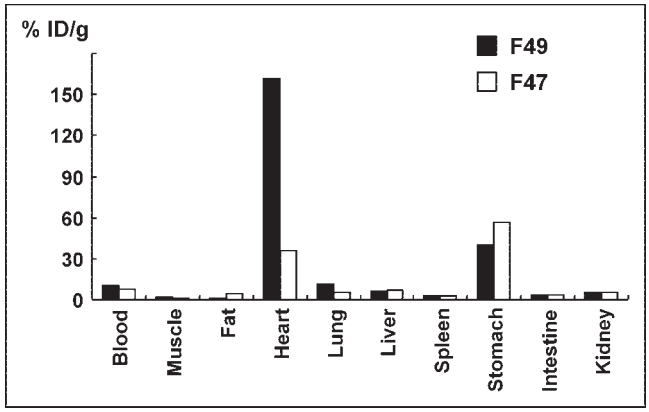

FIGURE 5. Biodistribution data of 2 transgenic mice that showed highest (F49) and lowest (F47) RUR_{heart} after injection of ^{99m}Tc-pertechnetate. Hearts show high uptake of radioisotope by expression of pαMHC-hNIS.

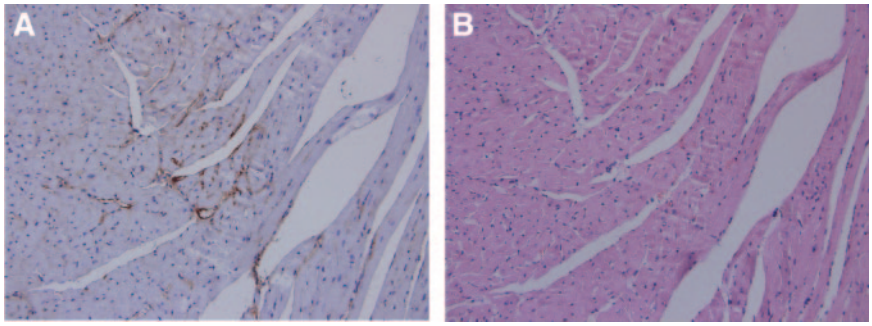

FIGURE 6. Immunohistochemical staining for NIS expression (A) and hematoxylin–eosin staining (B) (× 400). Brown staining along cytoplasmic membrane indicates extensive expression of NIS. However, normal histology of myocardium was confirmed.

protein was localized on the cytoplasmic membrane of cardiomyocytes. The hearts of transgenic mice were grossly normal on the histologic examinations.

DISCUSSION

In this study, we produced transgenic mouse lines with NIS expression driven by α-MHC promoter that was expressed specifically in cardiomyocytes (*12*). Although the expression of NIS in cardiomyocytes was somewhat heterogeneous, NIS expression in transgenic mice was specific to cardiomyocytes, as shown on γ-camera and microPET images. However, the heterogeneity of transgene expression is a usual phenomenon in the transgenic animal development because of the difference in the introduced copy number to genomic DNA, the difference in the incorporated site in genomic DNA, and the difference in the generation of the animal. The heterogeneity in this study was not a big problem if we consider such heterogeneity. Functionally normal NIS activity was proven by complete inhibition by $KClO_4$ administration. Inhibition by $KClO_4$ was observed simultaneously in both the thyroid gland and the heart.

Unlike the in vitro characteristics in some cancer cells transfected with NIS, which showed transient uptake and rapid efflux of radioiodine by NIS (*13,14*), the myocardium showed sustained uptake on 1-h dynamic images. The mechanism of longer retention of radioiodine in pαMHC-hNIS–expressing cardiomyocytes is unknown, but this provides the benefit of easy imaging and quantification.

The high uptake of the myocardium in transgenic mice is interesting in that all cells over the body of these mice have the transfected genome (NIS), but the α-MHC promoter gives the specificity of transgene expression only in cardiomyocytes. Because the NIS activity was sustained throughout the lifetime (up to 4-mo old) until sacrifice, cardiomyocyte-specific reporter transgene expression can be exploited for a transplantation–transdifferentiation study. We speculate that we can image differentiation into cardiomyocytes if the transgenic mice are used as donors of stem or progenitor cells in cardiac stem cell transplantation. Further investigation is in progress.

The merit of the transgenic mice was the ability to take in vivo images repeatedly on a γ-camera using ^{131}I or ^{99m}Tc-pertechnetate as a radiotracer. Furthermore, microPET imaging was reported recently using ^{124}I and small animals of NIS reporter transgenes (*15,16*). In general, PET yields a better resolution, sensitivity, and potential for quantitative analysis than γ-camera imaging. However, the resolution of microPET using ^{124}I still remains a problem (*17*). We could not show better resolution of ^{124}I microPET than pinhole γ-camera imaging using ^{99m}Tc-pertechnetate or ^{131}I in this study.

Cell transplantation therapy has been tried in various cardiac diseases using fetal cardiomyocytes, skeletal myoblasts, and autologous bone marrow cells (*18–20*). Only a small portion of transplanted cells are homing to the recipient's heart and differentiate into the cardiomyocytes. The desired route of administration and the best source of the stem or progenitor cells remain to be determined (*21*). Even after intracoronary administration of stem or progenitor cells, only a small percentage of cells was reported to reside in the injured myocardium (*22*). To track the transplanted cells, to monitor the cellular differentiation according to the time course, an imaging study in living animal is the best way. However, imaging methods to investigate the differentiation of transplanted cells have not been possible until now. The transgenic mouse model developed in this study can be used for such investigations because NIS reporter gene is expressed only in differentiated cardiomyocytes.

Several researchers have monitored the localization of transplanted cell by reporter genes (*9,23*) or MR agents (*24,25*). In vitro handling of the cells is mandatory for MRI and there is still doubt that the MR probes can be transferred to the daughter cells even if the stem cells have proliferated. When reporter genes were inserted into the stem or progenitor cells, the stability of the transgenes was also a problem (*26,27*). The sustainability of transgene expression of these mice, at least until 4-mo old, was a good alternative for tracking cells from allo- or xenograft transgenic mouse sources.

CONCLUSION

Our pαMHC-hNIS transgenic mice showed cardiomyocyte-specific and stable expression of the reporter gene, NIS, and may be a desirable donor in the study of cardiac cell transplantation. Although easy in vitro and in vivo monitoring methods for cellular differentiation are required,

our transgenic mice could provide a valuable source to study the differentiation of cardiomyocytes and contribute to the development of optimal cell-based therapy in various heart diseases.

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