Transfer of the Human NaI Symporter Gene Enhances Iodide Uptake in Hepatoma Cells

Uwe Haberkorn, Markus Henze, Annette Altmann, Shiming Jiang, Iris Morr, Miriam Mahmut, Peter Peschke, Wolfgang Kübler, Jürgen Debus, and Michael Eisenhut

Department of Nuclear Medicine, University of Heidelberg, Heidelberg; and Clinical Cooperation Units of Nuclear Medicine and Radiation Therapy, German Cancer Research Center, Heidelberg, Germany

The characteristic feature of thyroid cells of taking up iodide enables benign thyroid diseases and differentiated thyroid carcinoma to be successfully treated with radioiodide therapy. The transport of iodide across the cell membrane is mediated by the human NaI symporter (hNIS). We therefore investigated whether the accumulation of iodide may be induced by the retroviral transfer of the hNIS gene in nonthyroid tumor cells. **Methods:** With use of a bicistronic retroviral vector for the transfer of the hNIS coding sequence and the hygromycin resistance gene, rat Morris hepatoma (MH3924A) cells were infected with retroviral particles and 32 hNIS-expressing cell lines were generated by hygromycin selection. After incubation of the genetically modified and wild-type hepatoma cells and the rat thyroid cell line FRTL5 with $Na^{125}I$, the uptake and efflux of iodide were determined. In addition, the iodide distribution in rats bearing wild-type and genetically modified hepatomas was monitored. **Results:** Genetically modified MH3924A cell lines accumulated up to 235 times more iodide than did noninfected hepatoma cells. The maximal iodide uptake in the cells was observed after 60 min incubation time. Competition experiments in the presence of sodium perchlorate revealed a dose-dependent decrease of iodide uptake (87%–92%). Moreover, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone led to a loss of accumulated I^- (32%), whereas 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene increased the I^- uptake into the cells (22%). However, a rapid efflux of the radioactivity (80%) was observed during the first 10 min after $^{125}I^-$-containing medium had been replaced by nonradioactive medium. In rats, the hNIS-expressing tumors accumulated six times more iodide than did the contralateral wild-type tumor as monitored by scintigraphy. The ex vivo quantitation of the iodide content performed 1 h after tracer administration in 1g of tumor tissue revealed a 17-fold higher iodide accumulation in the genetically modified tumors. In accordance with the in vitro data, we also observed a rapid efflux of radioactivity from the tumor in vivo. **Conclusion:** The transduction of the hNIS gene per se is sufficient to induce $^{125}I^-$ transport in Morris hepatoma cells in vitro and in vivo. With regard to a therapeutic application, however, additional conditions need to be defined that inhibit the iodide efflux from the tumor cells.

Received Apr. 19, 2000; revision accepted Aug. 14, 2000.

For correspondence or reprints contact: Uwe Haberkorn, MD, Department of Nuclear Medicine, University of Heidelberg, Im Neuenheimer Feld 400, FRG-69120 Heidelberg, Germany.

Key Words: human NaI transporter; gene therapy; iodide uptake; hepatoma

J Nucl Med 2001; 42:317–325

The characteristic feature of thyroid follicular cells of accumulating iodide enables benign thyroid diseases and differentiated thyroid carcinoma to be successfully treated with radioiodide therapy. The complex process of iodide trapping in the thyroid tissue is initiated by the active transport of iodide and sodium ions into follicular cells, as mediated by the NaI symporter gene product. The energy-dependent transport proceeds against an electrochemical gradient, is coupled to the action of Na^+/K^+–adenosine triphosphatase, and is stimulated by thyroid-stimulating hormone (*1–5*). Recently, several experimental and clinical studies focused on expression of the NaI symporter (hNIS) gene in normal tissues and thyroid carcinomas of humans and animals, and the reduced radioiodide concentrating activity that is often observed in these tumors was attributed to a decreased expression of the NaI symporter gene (*6–12*). In patients with thyroid carcinomas, the reduced hNIS gene expression was shown to be associated with a downregulated expression of the thyroid peroxidase gene, thyroglobulin gene, and thyroid-stimulating hormone receptor gene (*6–12*). Moreover, a suggestion was made that the probability of thyroid carcinomas to take up iodide might be predicted by the immunologic quantitation of the hNIS expression (*9*).

The transcriptional downregulation of hNIS gene expression in thyroid carcinoma was supposed to be caused by methylation of the DNA sequence in critical regulatory regions and was reversed by the application of chemical demethylation (*12*). In some thyroid carcinoma cell lines, at least the hNIS mRNA expression or even the iodide transport was restored by treatment with 5-azacytidine or sodium butyrate, which induce demethylation of hNIS DNA in the untranslated region within the first exon (*12*). In follicular thyroid carcinoma cell lines, treatment with retinoic acid increased the hNIS expression, whereas no effect were observed in anaplastic thyroid carcinoma cells (*13*).

Using the rat Morris hepatoma cell line MH3924A as an in vitro–in vivo system, we investigated the effect of the retroviral transfer of the hNIS gene on iodide accumulation in carcinoma cells. This type of gene therapy can resolve the problem of inefficient transduction encountered with currently used viral vectors by the accumulation of radioactive isotopes with β emission. In this case, centers of trapping in the tumor can create a cross fire of β particles, thereby efficiently killing both the transduced and the nontransduced tumor cells. We report here that the transduction of the hNIS coding sequence is sufficient to induce iodide uptake, which, however, is associated with rapid efflux.

MATERIALS AND METHODS

Isolation of hNIS Gene and Construction of Bicistronic Retroviral Vector for Transfer of hNIS and Hygromycin Genes

A nested polymerase chain reaction (PCR) was performed to isolate the hNIS coding sequence from a human thyroid cDNA library (human thyroid gland QUICK-Clone cDNA; Clontech, Palo Alto, CA). Using the primers hNISoutfor (88–107) and hNISoutrev (2382–2403; the numbers refer to the nucleotide positions in the published sequence (*6*)), a PCR was performed in 30 cycles at the following conditions: 2.5 U Taq DNA (Boehringer Mannheim, Mannheim, Germany), deoxyribonucleoside triphosphate at a concentration of 200 μmol/L, primer at a concentration of 1 μmol/L, 1.5 mmol/L $MgCl_2$, and 10% dimethyl sulfoxide in a total reaction volume of 50 μL. The denaturation was done at 95°C for 1 min, the annealing at 58°C for 1 min, and the primer extension at 74°C for 2 min. Using the first PCR product as template and the primers hNISintfor (151–170) and hNISintrev (2363–2387), a second PCR at almost identical conditions was performed. During this PCR, 35 cycles were done at an annealing temperature of 66°C, and a 10-min final extension time was done at 74°C.

The PCR product was cloned into pCRScript (Stratagene, Heidelberg, Germany). The sequencing according to Sanger revealed three silent mutations at nucleotides 828 (C to T), 1,194 (G to A), and 1,200 (C to T) and, at nucleotide 323, a change C by T resulting in an amino acid replacement of A by V.

For transfer of the hNIS gene, a bicistronic retroviral vector based on the M48nlslacZ vector (*14*; obtained from O. Danos) was constructed: the hNIS gene and the hygromycin resistance gene that was taken from pIRES1hyg (Clontech) were cloned behind the elongation factor 1α (EF1α) promoter taken from the pShooter vector pEF/myc/cyto (Invitrogen; Groningen, The Netherlands) (Fig. 1). To ensure simultaneous expression of the genes coding for the hNIS and for the hygromycin resistance and stabilization of the mRNA, a synthetic intron and an internal ribosomal entry site from encephalomyocarditis virus were inserted between the genes.

Cell Culture, Retroviral Infection, and Generation of Recombinant Cell Lines

The rat Morris hepatoma cell line MH3924A and the transient packaging cell line BOSC23 (*15*) used for the production of ecotropic retroviral particles were cultured in RPMI1640 medium (Gibco BRL, Eggenstein, Germany) supplemented with 292 mg/mL glutamine, 100,000 IU/L penicillin, 100 mg/L streptomycin, and 20% fetal calf serum. The FRTL5 cell line, an iodide-concentrating rat thyroid cell line, was maintained in Ham's F12 medium modified by insulin (10 μg/mL), hydrocortisone (10 nU), transferrin (5 μg/mL), somatostatin (10 ng/mL), glycyl-histidyl-lysin (10 ng/mL), 5% fetal calf serum, and thyrotropin (1 U/100 mL). The cell lines were cultured at 37°C, in an atmosphere of 95% air and 5% CO_2.

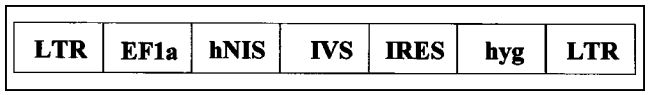

FIGURE 1. Structure of recombinant retroviral vector based on Moloney murine leukemia virus. This vector is designed to transfer human hNIS and hygromycin resistance (hyg) activity by use of internal ribosomal entry site (IRES) from encephalomyocarditis virus. Synthetic intron (IVS) is inserted to stabilize mRNA. Expression of genes is regulated by elongation factor 1α gene (EF1a) promoter. LTR = long terminal repeat.

For transient packaging of the retroviral DNA, a lipofection of BOSC23 cells was done. After 2 d, the medium was centrifuged to remove detached BOSC23 cells and was used for the infection of the MH3924A cells in the presence of 8 μg/mL polybrene overnight. The cells were treated with 425 μg/mL hygromycin for 3 wk until resistant cell lines were established.

For Northern blot analysis, total cellular RNA was isolated with the acid guanidinium thiocyanate phenol chloroform method (*16*). Five-microgram aliquots of RNA were electrophoresed in 1% agarose-formaldehyde gels and blotted using nylon membranes (Hybond N; Amersham, Braunschweig, Germany) in 20× saline sodium citrate buffer. The hybridization was performed with the cDNA probes for the hNIS and with β-actin (*17*) according to the method described by Church and Gilbert (*18*). The probes were labeled with ^{32}P-deoxycytidine triphosphate by use of a random primer labeling system (Gibco BRL, Eggenstein, Germany). After hybridization, an autoradiography was done with X-OMAT AR films (Eastman Kodak Company, Rochester, NY) for 4 h (β-actin) or 24 h (hNIS).

Measurement and Modulation of ^{125}I Uptake and Efflux

The iodide uptake was determined in triplicates in a modified procedure described by Weiss et al. (*2*). In the presence of 74 kBq (2 μCi) $Na^{125}I$ (Amersham, Buchler, Germany), of 625.3 MBq/μg (16.9 mCi/μg) specific activity, 3.7 GBq (100 mCi)/mL radioactive concentration, and 99.3% radiochemical purity, wild-type MH3924A tumor cells and recombinant cell lines were incubated for 4 h. After being washed twice with ice-cold phosphate-buffered saline, the cells were lysed with sodium hydroxide on ice. Alternatively, the cell lysis was performed in absolute ethanol and revealed the same uptake values. Thereafter, cell lysis with sodium hydroxide was used for the following experiments. Using an automated NaI(Tl) well counter (Cobra II; Canberra Packard, Meriden, CT) the radioactivity was measured in cell lysates and in the medium. The viable cell number was determined in a counter (Coulter Electronics, Dunstable, UK) and by trypan blue staining (more than 94% viable cells). Using the counter, a median cell volume of 1.78 fL (between 1.38 and 2.23 fL), with no significant difference for the genetically modified and wild-type MH3924A cells, was calculated.

To determine the iodide uptake in relation to the incubation time, the recombinant cell line hNIShyg10 and wild-type MH3924A cells were cultured with 74 kBq (2 μCi) $Na^{125}I$ for 1,

2, 5, 10, and 30 min or 1, 2, and 4 h. Washing and counting were performed as described.

For the modulation of the iodide uptake, hNIS-expressing and wild-type cells were incubated for 1 h in $Na^{125}I$ medium (74 kBq [2 μCi]) or $Na^{125}I$ medium supplemented with 10 or 50 μmol/L sodium perchlorate (Sigma, St. Louis, MO), 100 or 300 μmol/L of the anion channel blocker 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene (DIDS; Sigma), or 10 μmol/L of the proton conductor carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP; Sigma) according to Weiss et al. (*2*). Thereafter, the cells were washed, lysed, and counted as described.

To determine the ^{125}I efflux, recombinant and wild-type cells were incubated for 1 h in the presence or absence of 300 μmol/L DIDS with medium containing 74 kBq (2 μCi) $Na^{125}I$. After the cells had been washed twice, fresh nonradioactive medium was added. The cells were again incubated for 2, 4, 6, 8, 10, 12, 16, or 20 min and immediately lysed as described.

Measurement of ^{131}I Uptake in Tumor Tissue of Rats

The experiments were performed in compliance with German laws relating to the conduct of animal experimentation. Four $\times$ 10^6 tumor cells were transplanted subcutaneously into the right (hNIShyg10) or left (wild-type MH3924A) thigh of male young adult ACI rats weighing 200–250 g. For imaging studies, which were performed under general gaseous anesthesia (40% O_2:60% N_2O:1% halothane), only animals bearing tumors with a minimum size of 15 mm in diameter were accepted. Immediately after injection of 200 μL ^{131}I in 0.9% NaCl (14.8 MBq [400 μCi]) into the lateral tail vein of the rats, a dynamic scintigraphic image was taken for the following 60 min to evaluate radioactivity in the blood circulation, early tumor uptake, and the resulting body distribution of ^{131}I using a 25.4-cm (10-in.) scintillation camera (Searle-Siemens, Erlangen, Germany). The time-dependent relative accumulation of radioactivity in different regions of interest—for example, the heart, the liver, the tumor, the bladder, and the whole animal—was monitored in three animals 1, 2, 4 and 24 h after injection. To evaluate biologic stability of the transfected transporter genes, the experiment was repeated 1 wk later under identical conditions. The absolute amount of radioactivity (percentage injected dose per gram of wet tissue) was determined in another four animals, which were killed 1 h after injection to analyze the organs using a calibrated ionization chamber (Capintec, Inc., Ramsey, NJ).

RESULTS

Generation of hNIS-Expressing MH3924A Cell Lines

After infection of MH3924A cells with recombinant retroviruses and selection with hygromycin, 32 resistant cell lines were established. Fourteen of these lines were used for further experiments. Northern blot analysis revealed hNIS transcription in the cell lines hNIShyg1 to hNIShyg14. To investigate the functional activity of the recombinant hNIS gene product in the recombinant MH3924A cell line, iodide uptake experiments were performed.

$Na^{125}I$ Uptake and Efflux in Recombinant MH3924A Cell Lines

After the incubation with $Na^{125}I$ for 4 h, the hNIS-expressing hepatoma cell lines accumulated significantly more radiotracer than did the wild-type hepatoma cells or FRTL5 cells. The $^{125}I^-$ uptake of the FRTL5 cells was measured to be 50-fold higher than that of the wild-type hepatoma cells. However, with respect to the wild-type counterparts, between 84- and 235-fold more iodide was transported into the recombinant hepatoma cell lines (Fig. 2). The intracellular radioactivity was calculated to be up to 11.5% (cell line hNIShyg10) of the total radioactivity in cell lysate and medium. Given a median cell volume of 1.78 fL, an up to 10^5-fold higher concentration of radioactive iodide was observed in the cells than in the medium. The hNIShyg10 cells presented the maximal $^{125}I^-$ uptake and were therefore used for the following experiments. In Figure 3, the $^{125}I^-$ uptake in the hNIShyg10 cells in relation to the time of incubation with $Na^{125}I$ is shown. The initial uptake of iodide was dependent on incubation time, and cells accumulated $^{125}I^-$ maximally after 1 h incubation. The radioactivity measured after 2 and 4 h $^{125}I^-$ incubation was at a plateau, implicating steady-state uptake.

Figure 4 presents the effect of DIDS, FCCP, and sodium perchlorate on $Na^{125}I$ uptake in the hNIShyg10 cell line. In the presence of 10 or 50 μmol/L sodium perchlorate, we observed a dose-dependent inhibition (87% and 92%, respectively) of iodide accumulation in the hNIShyg10 cells. The addition of the proton conductor FCCP led to the loss of 32% of the accumulated I^-. The anion channel blocker DIDS had an opposite effect, as it increased the I^- uptake into the cells to 22% at a concentration of 300 μmol/L but not 100 μmol/L.

In the experiment presented in Figure 5, the iodide uptake was allowed to proceed for 1 h, at which time the steady-state level of accumulation was achieved. After the medium had been replaced by nonradioactive medium, the amount of $^{125}I^-$ in the hNIShyg10 cell lysates was determined as a function of time. Up to 77% of the cellular radioactivity was released into the medium after 10 min, and 90% efflux was observed after 20 min, indicating that the radiotracer was not trapped in the recombinant hepatoma cells. The addition of 300 μmol/L DIDS to the culture led to an increase in the initial $^{125}I^-$ uptake; however, a prolonged intracellular retention of the tracer was not achieved.

^{131}I Uptake in Morris Hepatoma in Rats

To investigate the iodide uptake in hNIS-expressing or -nonexpressing Morris hepatoma in vivo, hNIShyg10 cells or wild-type MH3924A cells were transplanted into the right and left thigh of male young adult ACI rats. After the animals had been injected with $^{131}I^-$, a dynamic scintigraphic image was obtained to evaluate radioactivity in the blood circulation, the tumor tissue, and different organs. Consistent with the data obtained from the in vitro studies, the hNIS-expressing tumor tissue significantly accumulated $^{131}I^-$, leading to scintigraphic visualization, whereas only low iodide uptake was observed in the wild-type hepatoma (Fig. 6). In the genetically modified tumor, the tracer accumulation increased to a maximum level during the first hour after administration, followed by a decrease of the intratumoral radioactivity (Fig. 7; Tables 1 and 2). Table 1 presents the ex vivo quantitation of the $^{131}I^-$ uptake per gram of

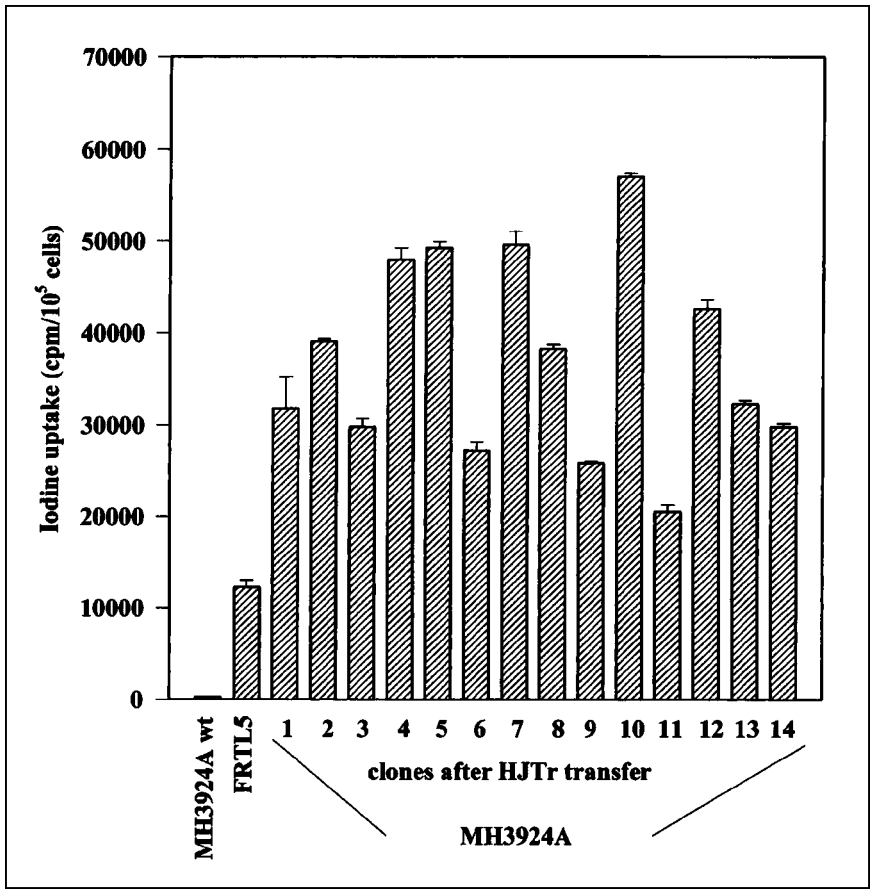

FIGURE 2. Iodide uptake in FRTL5 cells, wild-type Morris hepatoma cells, and hNIS-expressing Morris hepatoma cells after 4 h incubation with $Na^{125}I$. Data are mean values and SD ($n = 3$).

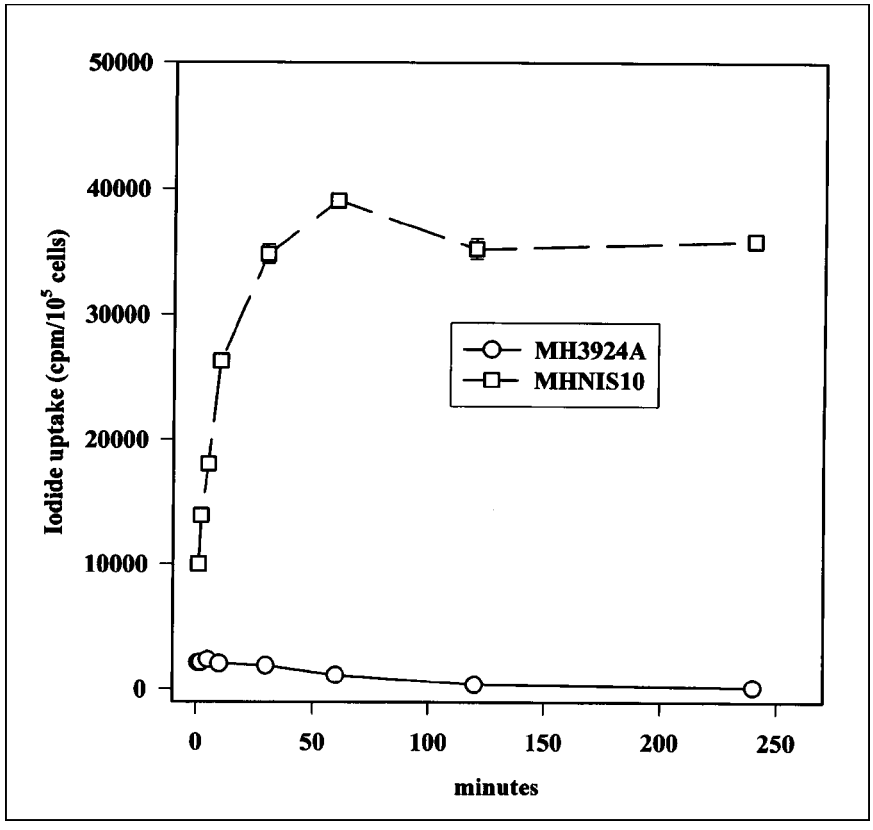

FIGURE 3. Time dependence of $^{125}I^-$ uptake in wild-type Morris hepatoma cells and in hNIS-expressing cell line hNIShyg10. Data are mean values and SD ($n = 3$).

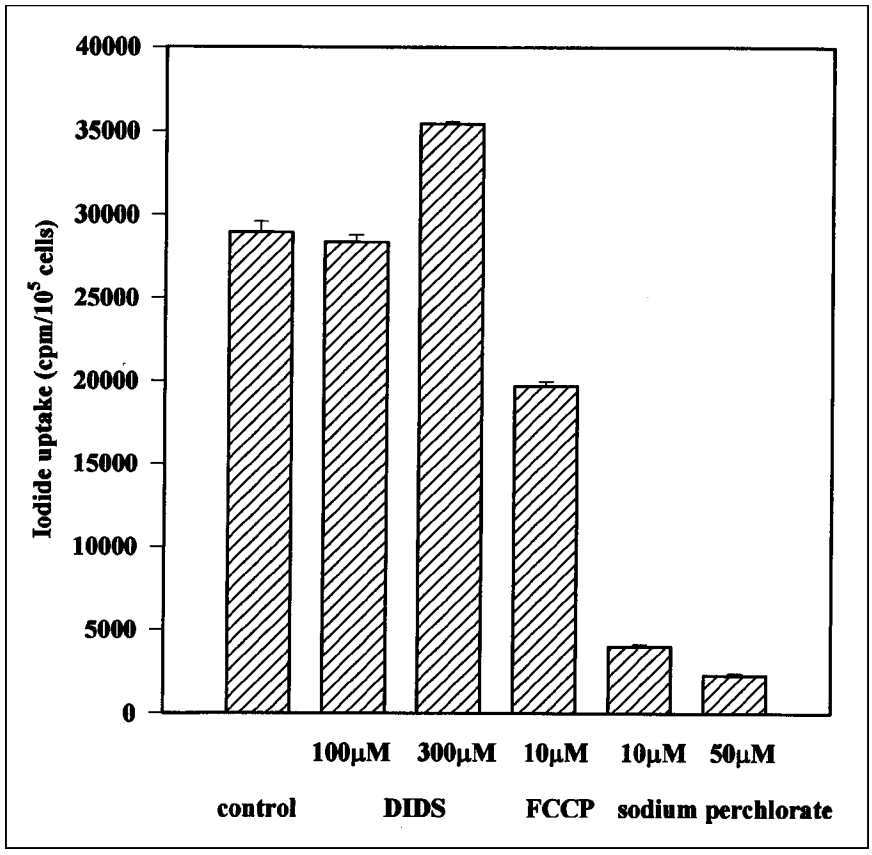

FIGURE 4. Effect of DIDS, FCCP, and sodium perchlorate on $^{125}I^-$ uptake in hNIShyg10 cell line after 1 h incubation. Data are mean values and SD (n = 3).

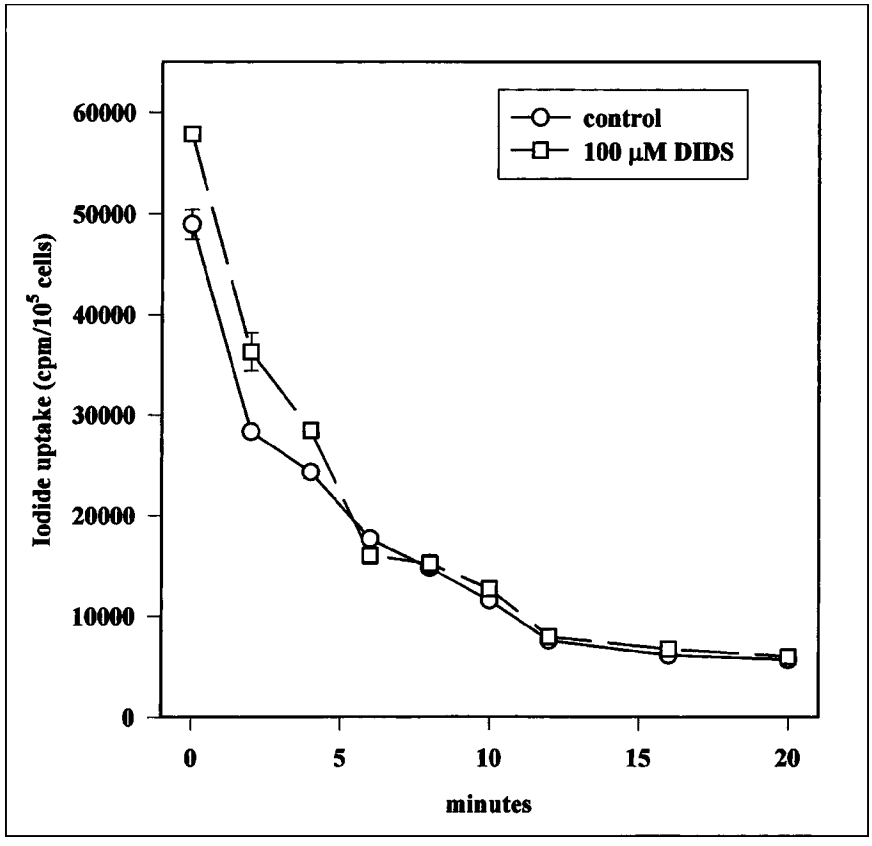

FIGURE 5. Iodide efflux from hNIShyg10 cells after 1 h incubation with $Na^{125}I$ with and without 300 μmol/L DIDS. Data are mean values and SD (n = 3).

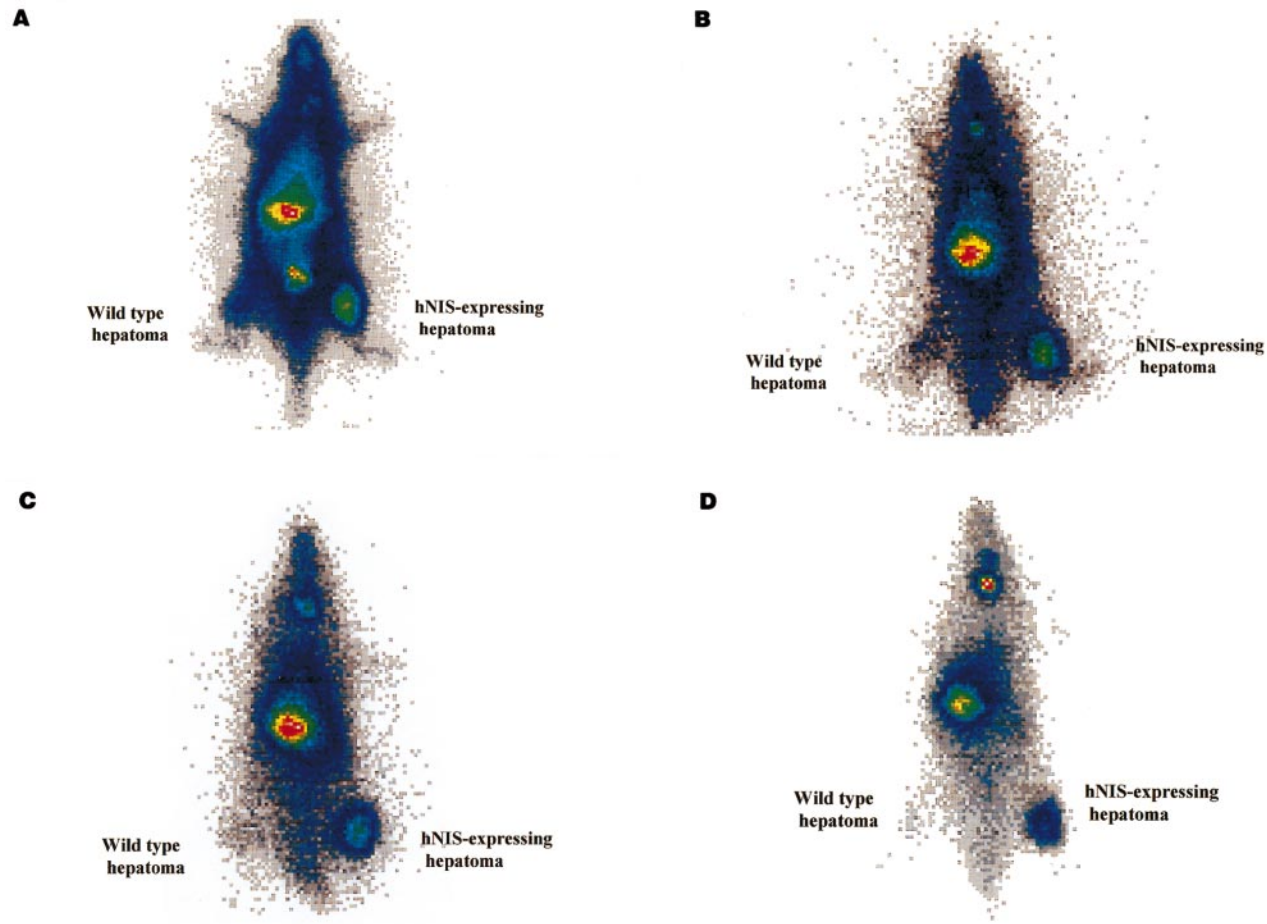

FIGURE 6. Scintigraphic images of tumor-bearing male young adult ACI rat with subcutaneously transplanted hNIS-expressing (right thigh) or wild-type Morris hepatoma cells (left thigh) at 30 min (A) and at 1 h (B), 2 h (C), and 4 h (D) after injection of $^{131}I^-$.

tissue from tumors and various organs 1 h after tracer administration. An up to 17-fold higher iodide accumulation was identified in the hNIS-expressing tumor tissues than in the wild-type tumors.

DISCUSSION

In this study, we investigated whether transduction of the gene coding for hNIS is sufficient to induce iodide uptake in Morris hepatoma (MH3924A) cells and tumor tissue in rats. Using a bicistronic retroviral vector for the hNIS and hygromycin resistance expression, we established MH3924A cell lines expressing the hNIS gene. With respect to the wild-type hepatoma cells and the rat FRTL5 thyroid cells that were used as controls, induction of iodide uptake was observed in all recombinant cell lines. The level of $^{125}I^-$ accumulation, however, varied significantly among individual cell lines (Fig. 2). Because integration of the retroviral genome occurs randomly at different sites of cellular DNA (*19*), we expected the genes coding for the hNIS and for hygromycin resistance to be expressed in variable amounts. The very high uptake (up to 235-fold more radiotracer compared with the wild-type cells) observed in our study can be attributed to the promoter used. The adult-type elongation factor 1α protein is one of the most abundant proteins in eukaryotic cells. Therefore, the DNA regulatory elements of the human elongation factor 1α gene serve as a strong constitutive promoter (*20,21*). Given a median cell volume of 1.78 fL calculated for the recombinant Morris hepatoma cells, and using tracer amounts of $Na^{125}I$, the radioactive iodide in the cells was up to 10^5-fold more than that in the medium. This amount exceeds by far the iodide concentration gradient of the FRTL5 cells and the thyroid gland. This gradient has been described to be 30-fold and 20- to 40-fold in vivo, respectively (*2,22*).

Studies concerning iodide uptake in the hNIShyg10 cell line relative to the period of incubation with $^{125}I^-$ revealed a maximal accumulation after 1 h (Fig. 3). In FRTL5 cells transfected with the rat NaI symporter gene expressed by a eukaryotic vector, a rapid iodide uptake at a maximal level was reached after 40 min (*23*). In like manner, Cos 7 cells transiently infected with the hNIS expression vector pcDNA3 accumulated 10-fold more $Na^{125}I$ than did control cells (*6*). Mandell et al. (*24*) modified human melanoma, mouse colon carcinoma, and human ovarian adenocarci-

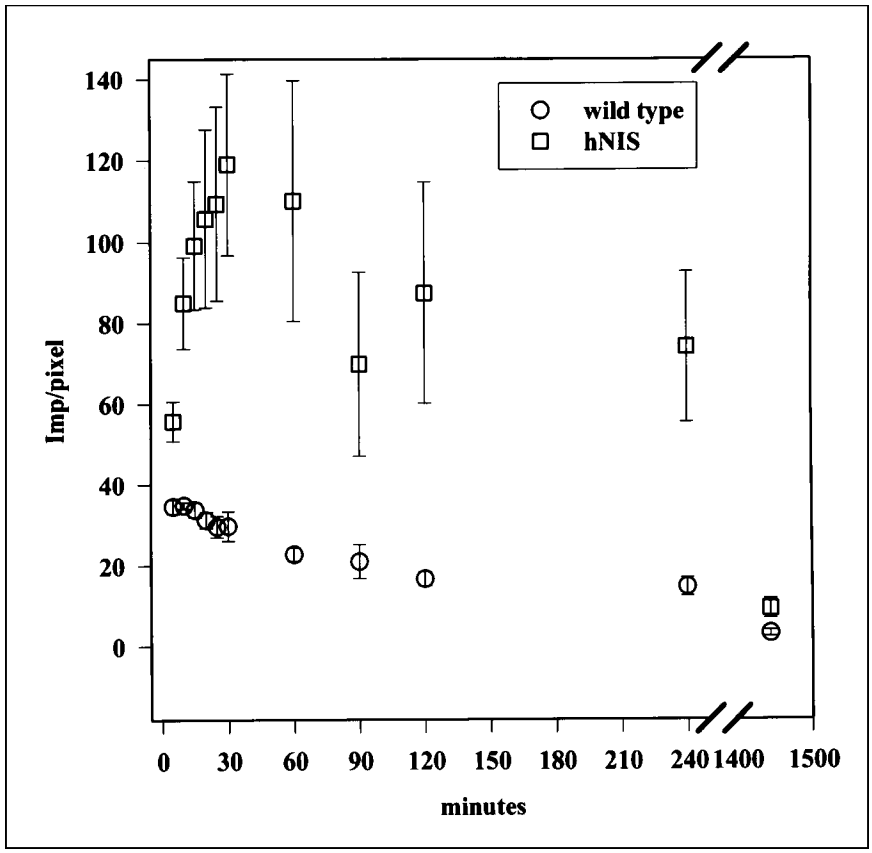

FIGURE 7. $^{131}I^-$ uptake (impulses [Imp]/pixel) in hNIS-expressing and wild-type Morris hepatomas at different times after radiotracer application in rats. Data are mean values and SD ($n = 3$).

noma cells with a retroviral vector bearing the rat NaI symporter gene and also observed an up to 35-fold increase in iodide uptake.

Anions such as SCN^- and ClO_4^- competitively inhibit the activity of hNIS, and the proton conductor FCCP has been described as preventing I^- uptake (*2*). To evaluate whether the iodide accumulation was specifically induced by the functional activity of the hNIS gene product, iodide uptake was determined in the hNIShyg10 cells in the presence of sodium perchlorate, the anion channel blocker DIDS, or the proton conductor FCCP. Iodide uptake was decreased in the presence of FCCP and also in a dose-dependent manner in the presence of sodium perchlorate, indicating that a functional hNIS is expressed in the genetically modified cell lines (Fig. 4). In contrast, the potent erythrocyte anion channel blocker DIDS, which has been described as stimulating the initial influx of iodide in the rat thyroid cell line FRTL5 (*2*) and in porcine thyroid cells (*5*),

TABLE 1
Iodide Activity Concentration 1 Hour After Injection of 14.8 MBq (400 μCi) ^{131}I

Tissue	Concentration (kBq/g of tissue)	Weight (g)
HNIS-expressing tumor	532.8 ± 88.8	1.1 ± 0.4
Wild-type tumor	29.6 ± 14.8	2.5 ± 1.3
Thyroid	3700 ± 1184	0.05 ± 0.02
Blood	77.1 ± 11.1	4.4 ± 1.4
Liver	37.0 ± 7.4	7.7 ± 0.9
Stomach	995.3 ± 469.9	3.4 ± 1.6
Kidney	40.7 ± 11.1	1.8 ± 0.1
Small bowel	51.8 ± 7.4	6.6 ± 2.1
Bladder	758.5 ± 247.9	1.4 ± 0.4
Whole animal	66.6 ± 7.4	239.7 ± 24.4

Data are mean values and SD ($n = 4$). Weight represents weight of tissue sample measured, including blood.

TABLE 2
Scintigraphic Uptake in hNIS-Expressing Tumors, Wild-Type Tumors, and Thyroid of Tumor-Bearing Rats

Time (min)	hNIS	Wild type	Thyroid	*n*
5	1.4 ± 0.2	1.0 ± 0.1	0.50 ± 0.01	7
10	2.1 ± 0.4	1.1 ± 0.2	0.51 ± 0.02	7
15	2.4 ± 0.4	1.0 ± 0.1	0.54 ± 0.04	7
20	2.5 ± 0.5	0.99 ± 0.1	0.54 ± 0.04	7
25	2.6 ± 0.5	0.95 ± 0.1	0.55 ± 0.06	7
30	2.7 ± 0.6	0.9 ± 0.1	0.56 ± 0.06	7
65	2.8 ± 0.6	0.8 ± 0.1	0.79 ± 0.05	7
120	2.7 ± 1.2	0.57 ± 0.1	1.10 ± 0.09	3
240	2.4 ± 0.9	0.57 ± 0.1	3.77 ± 0.65	3
1445	0.97 ± 0.2	0.40 ± 0.05	14.28 ± 1.12	3

Data are percentage of uptake in whole animal.

caused an increased uptake of 22% in the hNIShyg10 cell line.

The failure of thyroid tissues to accumulate radioiodide is usually associated with reduced hNIS gene expression in the cells. Some thyroid carcinomas and cell lines, however, fail to concentrate iodide although the hNIS gene is actively transcribed (*12*,*25*), implying that iodide accumulation is additionally influenced by factors distinct from expression of the hNIS gene. Besides transport into the cell, trapping of the radioiodide is necessary for therapeutic efficiency. To evaluate the amount of iodide trapped in tumor cells, efflux experiments were performed on the hNIShyg10 cell line. After the $^{125}I^-$-containing medium had been replaced by nonradioactive culture medium, a rapid efflux was observed within the next 10 min, indicating that no organification of the radioactive iodide had occurred (Fig. 5). Recently, iodide efflux was also described for NaI symporter–expressing FRTL-Tc cells, although the efflux was slower than in the wild-type FRTL-Tc cell line, in which the mechanism remains unknown (*23*,*24*).

Analogous to the cell culture system, maximal iodide uptake into the genetically modified hepatoma was achieved 1 h after the rats had received injections of $^{131}I^-$. Although the hNIS protein favors iodide transport into the cells rather than efflux, the radioactivity continuously disappeared from the hNIS-expressing tumors and from different organs of the body, except the thyroid gland, in which an increasing iodide uptake was seen with time because of iodide trapping (Figs. 6 and 7; Tables 1 and 2). Moreover, we calculated a shorter biologic $^{131}I^-$ half-life in hNIS-expressing tumors (14.5 $\pm$ 4.8 h) than in wild-type tumors (15 $\pm$ 4.9 h) within the first 24 h after tracer administration. We suggest that the decrease in intracellular iodide concentration is proportional to the total iodide clearance of the body. Consequently, we expect the exposure time of genetically modified tumor cells to $^{131}I^-$radiation to be too short for therapeutic relevance unless iodide is organified. Recently, selective killing of up to 64% of cells was observed in rat NaI symporter transduced tumor cells in vitro (*24*), but this effect was not reproduced in vivo (*23*). In rat FRTL tumors, a radiation dose of 4 Gy was calculated when a dose of approximately 37 MBq (1 mCi) ^{131}I had been injected, with an effective half-life of 6 h (*23*). Using MIRDOSE3 (*27*), we calculated a cumulated activity of 1,147.7 MBq $\times$ s and 20,080 MBq $\times$ s for the wild-type and the hNIS-expressing tumors, respectively. This activity resulted in an absorbed dose of 35 mGy (wild-type tumor) and 592 mGy (hNIS-expressing tumor) after administration of 14.8 MBq (0.4 mCi) ^{131}I. Previously, the half-life of radioiodide in human sera of patients on an iodine-depletion regimen was found to be 20 h (*23*,*26*), indicating that a higher radiation dose in human genetically modified tumors may be achieved by a longer iodide circulation time.

CONCLUSION

In conclusion, transduction of the hNIS gene induces accumulation of iodide in non–I^--concentrating hepatoma cells. However, the iodide is not organified in the genetically modified cells, because the high influx is followed by a rapid efflux in vitro and in vivo. With regard to a gene therapeutic approach based on transduction of the hNIS gene, studies on pharmacologic modulations interfering with iodide efflux from cells are currently being performed. On the other hand, transduction of the hNIS gene using a bicistronic vector may serve as a simple reporter system for visualization of other genes. In this respect, hNIS expression and the application of ^{99m}Tc-pertechnate may be used to monitor the expression of a second therapeutic gene in tumors (*28*).

ACKNOWLEDGMENTS

The authors thank Reiner Kühnlein for help with the animal experiments. This study was supported by a grant from the Tumorzentrum Heidelberg/Mannheim, Heidelburg, Germany.

REFERENCES

1. Marcocci C, Cohen JL, Grollman EF. Effect of actinomycin D on iodide transport in FRTL-5 thyroid cells. *Endocrinology.* 1984;115:2123–2132.
2. Weiss SJ, Philp NJ, Grollman EF. Iodide transport in a continuous line of cultured cells from rat thyroid. *Endocrinology.* 1984;114:1090–1098.
3. Paire A, Bernier-Valentin F, Selmi-Ruby S, Rousset B. Characterization of the rat thyroid iodide transporter using anti-peptide antibodies. *J Biol Chem.* 1997;272: 18245–18249.
4. Nakamura Y, Ohtaki S, Yamazaki I. Molecular mechanism of iodide transport by thyroid plasmalemmal vesicles: cooperative sodium activation and asymmetrical affinities for the ions on the outside and inside of the vesicles. *J Biochem.* 1988;104:544–549.
5. Nakamura Y, Kotani T, Ohtaki S. Transcellular iodide transport and iodination on the apical plasma membrane by monolayer porcine thyroid cells cultured on collagen-coated fibers. *J Endocrinol.* 1990;126:275–281
6. Smanik PA, Liu Q, Furminger TL, et al. Cloning of the human sodium iodide symporter. *Biochem Biophys Res Commun.* 1996;226:339–345.
7. Cho JY, Sagartz JE, Capen CC, Mazzaferri EL, Jhiang SM. Early cellular abnormalities induced by RET/PTC1 oncogene in thyroid-targeted transgenic mice. *Oncogene.* 1999;18:3659–3665.
8. Arturi F, Russo D, Schlumberger M, et al. Iodide symporter gene expression in human thyroid tumors. *J Clin Endocrinol Metab.* 1998;83:2493–2496.
9. Caillou B, Troalen F, Baudin E, et al. Na^+/I^- symporter distribution in human thyroid tissues: an immunohistochemical study. *J Clin Endocrinol Metab.* 1998; 83:4102–4106.
10. Ryu KY, Senokozlieff ME, Smanik PA, et al. Development of reverse transcription-competitive polymerase chain reaction method to quantitate the expression levels of human sodium iodide symporter. *Thyroid.* 1999;9:405–409.
11. Lazar V, Bidart JM, Caillou B, et al. Expression of the Na^+/I^- symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes. *J Clin Endocrinol Metab.* 1999;84:3228–3234.
12. Venkataraman GM, Yatin M, Marcinek R, Ain KB. Restoration of iodide uptake in dedifferentiated thyroid carcinoma: relationship to human Na^+/I^- symporter gene methylation status. *J Clin Endocrinol Metab.* 1999;84:2449–2457.
13. Schmutzler C, Winzer R, Meissner-Weigl J, Kohrle J. Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. *Biochem Biophys Res Commun.* 1997;240:832–838.
14. Moullier P, Marechal V, Danos O, Heard JM. Continuous systemic secretion of a lysosomal enzyme by genetically modified mouse skin fibroblasts. *Transplantation.* 1993;56:427–432.
15. Pear WS, Nolan GP, Scott ML, Baltimorre D. Production of high titer helper-free retroviruses by transient transfection. *Proc Natl Acad Sci USA.* 1993;90:8392–8396.

16. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162:156–159.
17. Cleveland DW, Lopata MA, MacDonald RJ, Cowan NJ, Rutter WJ, Kirschner MW. Number and evolutionary conservation of D- and E-tubulin and cytoplasmic E- and F-actin genes using specific cloned cDNA probes. *Cell.* 1980;20:95–105.
18. Church GM, Gilbert W. Genomic sequencing. *Proc Natl Acad Sci USA.* 1984; 81:1991–1995.
19. Goff SP. Genetics of retroviral integration. *Ann Rev Genet.* 1992;26:527–544.
20. Mizushima S, Nagata S. pEF-BOS, a powerful mammalian expression vector. *Nucleic Acids Res.* 1990;18:5322.
21. Wakabayashi-Ito N, Nagata S. Characterization of the regulatory elements in the promoter of the human elongation factor-1α gene. *J Biol Chem.* 1994;269:29831–29837.
22. Kaminsky SM, Levy O, Salvador C, Dai G, Carrasco N. Na^+I^- symport activity is present in membrane vesicles from thyrotropin-deprived non-I^- transporting cultured thyroid cells. *Proc Natl Acad Sci USA.* 1994;91:3789–3793.
23. Shimura H, Haraguchi K, Miyazaki A, Endo T, Onaya T. Iodide uptake and experimental ^{131}I therapy in transplanted undifferentiated thyroid cancer cells expressing the Na^+/I^- symporter gene. *Endocrinology.* 1997;138:4493–4496.
24. Mandell RB, Mandell LZ, Link CJ. Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. *Cancer Res.* 1999;59:661–668.
25. Venkataraman GM, Yatin M, Ain KB. Cloning of the human sodium-iodide symporter promoter and characterization in a differentiated human thyroid cell line, KAT-50. *Thyroid.* 1998;8:63–69.
26. Maruca J, Santner S, Miller K, Santen RJ. Prolonged iodine clearance with a depletion regimen for thyroid carcinoma. *J Nucl Med.* 1984;25:1089–1093.
27. Stabin M. MIRDOSE: personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med.* 1996;37:538–546.
28. Gambhir SS, Barrio JR, Herschman HR, Phelps ME. Assays for noninvasive imaging of reporter gene expression. *Nucl Med Biol.* 1999;26:481–490.