

Sodium Iodide Symporter: Its Role in Nuclear Medicine*

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Thyroid iodide uptake is basic to the clinical applications of radioiodine. Iodide uptake occurs across the membrane of the thyroid follicular cells through an active transporter process mediated by the sodium iodide symporter (NIS). The recent cloning of the NIS gene enabled the better characterization of the molecular mechanisms underlying iodide transport, thus opening the way to the clarification and expansion of its role in nuclear medicine. In papillary and follicular carcinoma, NIS immunostaining was positive in only a few tumor cells, and no NIS protein expression was detected in anaplastic carcinomas. Decreased NIS expression levels account for the reduced iodide uptake in thyroid carcinomas. Thus, by targeting NIS expression in cancer cells, we could enable these cells to concentrate iodide from plasma and in so doing offer the possibility of radioiodine therapy. Several investigators have shown that gene transfer of NIS into a variety of cell types confers increased radioiodine uptake by up to several hundredfold that of controls in nonthyroid cancers as well as in thyroid cancer. In addition, my group proposes that NIS may serve as an alternative imaging reporter gene in addition to the HSVtk and dopaminergic receptor genes. The NIS has the potential to expand the role of nuclear medicine in the future, just as it has served as the base for the development of nuclear medicine in the past.

Key Words: sodium iodide symporter; thyroid carcinoma; gene therapy; reporter gene

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Iodine represents an essential element in thyroid physiology, as it is a critical component of the thyroxine and triiodothyronine molecules. The concentration of iodide in the thyroid gland is the first step in the production of thyroid hormones, and it was first reported as early as 1915. The thyroid gland was found to be capable of concentrating iodide by a factor of 20–40 with respect to its plasma level (1).

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Radioactive isotopes of iodine were first used as tracers of thyroid function and, subsequently, for the treatment of hyperthyroidism and benign thyroid diseases. In addition, evidence of the important role of iodine transport in thyroid cancer cells provided the basis for the use of radioiodine for the diagnosis and treatment of thyroid cancer (2). Radioiodine was first used for thyroid cancer treatment in 1941, became widely available after the World War, and led to the birth of nuclear medicine (3). Currently, this radioiodide-concentrating activity still has important clinical applications for the evaluation, diagnosis, and treatment of various thyroid diseases, including thyroid cancers, and remains one of the main clinical applications of nuclear medicine.

In addition, ^{99m}Tc -pertechnetate has also been commonly used to image the thyroid gland. It is transported to thyrocytes by the same mechanism that transports and concentrates radioiodine in the thyroid gland.

DISCOVERY OF NIS AND ITS GENE

Work in the early 1960s established that thyroid iodide transport, previously referred to as the iodide pump or the iodide trap, is saturable and specific. It was subsequently shown that the driving force for iodide uptake against the electric gradient is the transmembrane sodium ion concentration gradient generated and maintained by the sodium-potassium adenosine triphosphatase. The iodide pump itself is actually an NIS, which belongs to the sodium/glucose cotransporter family (4).

A thorough biochemical, immunologic, and electrochemical characterization of rat NIS (rNIS) was published by Carrasco in 1996 (2,5). The cloning of rNIS by her group represented a major step forward. Later, Jhiang et al., using the complementary DNA (cDNA) sequence of the rat iodide transporter, isolated and cloned the human homolog (6). The nucleotide sequence of human NIS (hNIS) cDNA, encompassing the coding region, was determined from a partial cDNA clone isolated from a human thyroid cDNA library and extended by rapid amplification of 3' cDNA ends (3'-RACE) analysis, with an overlap of 18 amino acids between the 2 cDNA fragments.

The identification of the hNIS gene will enable further investigation of the complex mechanisms regulating iodide uptake activity in the thyroid, open a new avenue of diag-

nostic and therapeutic opportunity for NIS, and extend its use in nuclear medicine research and clinical practice (6).

STRUCTURE AND FUNCTION OF NIS

Structure

The hNIS gene is localized on chromosome 9p12-13.2 and encodes a glycoprotein of 643 amino acids with a molecular mass of approximately 70–90 kDa. The coding region of hNIS contains 15 exons interrupted by 14 introns and codes for a 3.9-kb messenger RNA (mRNA) transcript. As a member of the sodium-dependent transporter family, NIS is an intrinsic membrane protein with 13 putative transmembrane domains, an extracellular amino-terminus, and an intracellular carboxyl-terminus. The NIS protein has 3 potential *N*-linked glycosylation sites; one is located in the 7th extramembranous domain (the 4th extracellular loop), and 2 are located in the 13th extramembranous domain (the last extracellular loop) (7).

The amino acid sequence encoded by hNIS shows 84% identity (92% similarity) to rNIS. Most of the nonhomologous amino acids of human and rNIS are located in the putative carboxyl-terminal cytoplasmic domain and in the extracellular loop between transmembrane domains XI and XII, where hNIS contains an additional 5 amino acids. The carboxyl-terminus of hNIS contains a large hydrophilic region of 94 amino acids, which is 20 amino acids longer than the carboxyl-terminal domain of rNIS (6).

Function

NIS is an integral protein of the basolateral membrane of thyroid gland follicular cells (5). The NIS-catalyzed active accumulation of iodide from the interstitium into the cell is achieved against an electrochemical gradient. NIS couples the energy released by the inward “downhill” translocation of Na^+ along its electrochemical gradient, generated by sodium–potassium adenosine triphosphatase, to the energy driving the simultaneous inward “uphill” translocation of iodide against its electrochemical gradient (8). NIS cotransports 2 sodium ions and 1 iodide ion, and the transmembrane sodium gradient serves as the driving force for iodide uptake (Fig. 1). NIS-mediated iodide transport is inhibited by the sodium–potassium adenosine triphosphatase inhibitor ouabain and by the competitive inhibitors thiocyanate and perchlorate. After active transport across the basolateral membrane of the thyroid follicular cells, iodide is translocated across the apical membrane by pendrin, the Pendred’s syndrome gene product, which is a chloride/iodide transporter (9).

In addition to iodide, several other anions are transported by NIS: $\text{I}^- = \text{SeCN}^- > \text{SCN}^- > \text{ClO}_3^- > \text{NO}_3^-$. NIS also transports technetium pertechnetate and rhenium perrhenate. The only apparent common denominator of these well-transported substrates is anionic monovalency. Moreover, the closer the size of the monovalent anion to that of iodide ion, the better it is transported (5). In addition, the specificity of sodium-dependent cotransporters for the so-

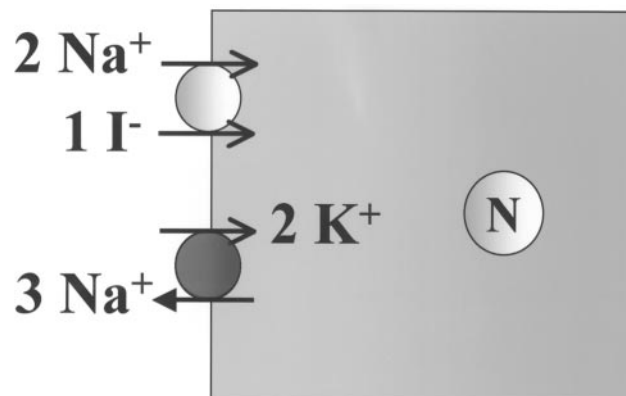


FIGURE 1. Schematic illustration of NIS. NIS cotransports 2 sodium ions and 1 iodide ion. Sodium gradient provides energy for this transfer, as generated by Na^+/K^+ -adenosine triphosphatase.

dium ion as a driving cation is not absolute. Iodide transport through NIS is not driven by H^+ , but Li^+ is able to drive transport at a reduced level (10%–20% of Na^+ -driven transport) (5).

Regulation

Many studies, using different experimental models, have elucidated the role of thyroid-stimulating hormone (TSH) and the activation of the cyclic adenosine monophosphate (cAMP) pathway as the principal regulator of iodide uptake. Many other factors, including insulin, insulinlike growth factor I, epidermal growth factor, and iodide itself, also influence iodide uptake in the thyroid gland (8).

TSH increases radioiodine uptake in thyroid cancer cells and in normal thyrocytes, as seen in radioiodine whole-body scans. Thyroid hormone is withdrawn for 4–6 wk or human recombinant TSH is injected before radioiodine whole-body scanning. Early observations made before the isolation of NIS cDNA suggested that TSH stimulated the iodide accumulation results, at least in part, from the cAMP-mediated increased biosynthesis of NIS. Using high-affinity anti-NIS antibody, Levy et al. (10) showed in rats that NIS protein expression is upregulated by TSH in vivo. TSH was found to upregulate NIS gene expression and NIS protein abundance, by some 2.5- to 2.7-fold after 24 h and 48 h, respectively (8). However, iodide uptake was enhanced 27-fold, and an increase was evident after 24-h stimulation. Taken together, these observations suggest that other mechanisms, including cAMP-dependent phosphorylation and the participation of other activating or inhibiting proteins, may be involved in the posttranslational regulation of NIS activity.

The treatment of small to moderate amounts of iodide does not influence the uptake of simultaneously administered ^{131}I by the thyroid. However, as the iodide doses become progressively larger, organic bindings are inhibited. The decreasing yield of organic iodine from increasing doses of inorganic iodide is termed the acute Wolff–Chaikoff effect. The regulatory role played by iodide on

NIS function was explored at the molecular level after NIS cDNA was isolated. In vivo studies have shown that iodide inhibits the expression of both thyroid peroxidase (TPO) and NIS mRNAs in the dog thyroid, a finding consistent with the Wolff–Chaikoff effect (11). When a high serum iodide concentration (100–200 µg/dL) is maintained by the repeated administration of iodide, the inhibitory effect disappears and thyroid iodine organification increases. This is called escape from the Wolff–Chaikoff effect. Eng et al. (12) found decreased levels of NIS mRNA and NIS protein in response to both chronic and acute iodide excess in rats. They suggested the hypothesis that the escape from the acute Wolff–Chaikoff effect is caused by a decrease in NIS, which induced a resultant lowering of the intrathyroidal iodine content below a critical inhibitory threshold, thus allowing organification of the iodide to resume.

Interleukin-1, tumor necrosis factor, and interferon all inhibit TSH-stimulated hNIS gene expression and iodide uptake in thyrocyte cultures. This suggests that the inhibition of iodide uptake by these cytokines is mediated, at least partly, by the downregulation of NIS gene expression. Interleukin-1, tumor necrosis factor, and interferon are produced by inflammatory infiltrates and thyroid follicular cells in autoimmune thyroid disease and may therefore play a role in autoimmune hypothyroidism by downregulating hNIS mRNA expression. Similar mechanisms could also be implicated in the thyroid abnormalities associated with subacute thyroiditis and nonthyroidal illness (13). These mechanisms are also consistent with the finding of irregular decreased uptake of radioiodine in thyroiditis on thyroid scans.

NIS gene expression in thyroid cells is regulated by transcription factors, such as thyroid transcription factor 1, paired domain transcription factor, and NIS TSH-responsive factor-1, the expressions of which are enriched in the thyroid (14,15).

Normal Distribution of NIS

Thyroid Tissue. In normal thyroid tissue, follicular epithelial cells exhibit a heterogeneous pattern when stained with hNIS antibodies. Inside a given follicle, isolated follicular cells are strongly immunostained and are totally distinct from weakly positive or negative cells. Filetti et al. (8) reported that approximately 30% of cells express detectable amounts of hNIS protein. A different pattern was observed for the TSH receptor, which was constitutionally expressed in all normal follicular cells. In positive cells, hNIS staining was confined to the basolateral membrane. Stromal cells, lymphocytes, intrafollicular macrophages, and vascular endothelial cells did not react with the hNIS antibodies (4).

Nonthyroid Tissues. NIS mRNA is detected in many nonthyroid tissues, including the salivary glands, stomach, thymus, and breast. Lower levels of expression are detected in the prostate, ovary, adrenal gland, lung, and heart. By

contrast, the NIS gene has not been detected in the colon, orbital fibroblasts, or nasopharyngeal mucosa (8).

This is consistent with the finding that uptake of radioiodine or ^{99m}Tc-pertechnetate is almost always found in the salivary glands, stomach, lactating breast, and placenta. Occasionally, radioiodine uptake is also seen in nonlactating breasts. Radioiodine secretion in tears and the supraorbital uptake of ^{99m}Tc-pertechnetate in patients without eye disorders suggest that active iodide uptake might also occur in the lacrimal glands (16).

Salivary glands express hNIS protein. However, the immunostaining of NIS protein occurs only in ductal cells, where it is diffusely expressed within cells, and not within acinar cells. In the human stomach, expression of NIS is restricted to parietal cells (8). Immunostaining of the lactating mammary gland showed distinct NIS-specific immunoreactivity at the basolateral membrane of the alveolar epithelial cells. Examination of NIS protein expression at various physiologic stages showed that NIS is exclusively present in the mammary gland during gestation and lactation, which is in contrast to the constitutive expression of NIS in the thyroid gland (9).

Expression of NIS in normal mammary tissue seems to be driven by a combination of hormonal factors. Oxytocin, a hormone released during lactation, induces NIS expression and radioiodine transport, whereas prolactin, another hormone required for milk production, suppresses this effect. The antagonistic effect of prolactin on the oxytocin-induced increase of NIS expression may be due to the reported inhibitory effect of prolactin on steroidogenesis (17). In oophorectomized animals, a combination of estrogen, oxytocin, and prolactin led to maximal NIS expression, which resembled the relative hormonal levels observed during lactation, which causes the highest levels of NIS expression in the breast (9).

Spitzweg et al. (18) found that the nucleotide sequences of hNIS cDNA derived from the parotid gland, mammary gland, and gastric mucosa were identical to the hNIS cDNA sequence of the thyroid. However, TSH stimulates radioiodine uptake in thyroid tissues and not in these extrathyroidal tissues because of their absence of TSH receptors. Diminished iodide transport in extrathyroidal tissues may result from altered NIS gene transcriptional activity, perhaps as a consequence of altered promoter structure or function, or from altered NIS mRNA or protein turnover. Variable and lower NIS transcriptional activity in extrathyroidal tissues may be accounted for, at least in part, by thyroid-specific transcription factors that act on the NIS promoter to control NIS gene expression in the thyroid gland. Specific gene expression of thyroglobulin, thyroperoxidase, and TSH receptor in the thyroid gland is known to be regulated at the transcriptional level. Thyroid transcription factor 1, a homeodomain-containing protein, has been found to bind to all 3 thyroid-specific promoter regions. These findings suggest that thyroid transcription factor 1 may be one of the factors capable of activating NIS gene expression in the

thyroid gland and thus to account for the lower levels of NIS gene expression in extrathyroidal tissues (18).

Relation of NIS Gene to Other Genes

It is suggested that the expressions of NIS, thyroglobulin, TSH receptor, and TPO genes are regulated by distinct molecular mechanisms or, alternatively, that they may have a different pattern of sensitivity to the mechanisms of oncogenic transformation. Trapasso et al. (19) found similar discrepancies *in vitro*; that is, oncogene transformed rat thyroid cells lacking iodine uptake, whereas thyroglobulin, TPO, and TSH receptor gene expression were present to variable extents. The regulation of NIS expression at the mRNA level appears to be different from that of other thyroid-specific markers.

The reduced expression of potential novel nuclear factors may contribute to reduced NIS expression resulting in the absence of iodide uptake in some papillary thyroid cancers (20). Magliano et al. (21) demonstrated that the reintroduction of paired domain transcription factor in PCPy cells was sufficient to activate the expression of the endogenous genes encoding thyroglobulin, TPO, and NIS. Thus, this cell system provided direct evidence on the ability of paired domain transcription factor to activate transcription of thyroid-specific genes at their chromosomal locus and further strongly suggested a fundamental role for this transcription factor in the maintenance of functional differentiation in thyroid cells.

NIS mRNA expression levels have been shown to be decreased in oncogene-transformed PC Cl 3 rat thyroid cell lines (PC v-erbA, PC HaMSV, PC v-raf, and PC E1A) and to be almost completely absent in PC RET/PTC, PC KiMSV, PC p53^{143ala}, and PC PyMLV. These results suggest that oncogene activation may be involved in the pathophysiology of reduced NIS expression in thyroid cancer (19). Different oncogenic pathway activation (PyMLV, p53^{143ala}, *ras*) may produce the same effect, and the same oncogenic pathway (*v-ras* Ha, *v-ras* Ki) may produce different effects on the transcriptional regulation of the NIS gene.

Russo et al. (22) proposed that the activation of different proto-oncogenes may inhibit iodide uptake activity in thyroid tumor cells by decreasing NIS gene expression, as shown *in vitro* in rat thyroid cells transformed with different oncogenes and *in vivo* in transgenic mice expressing *ret/PTC1*, *ret/PTC3*, *TPK-T1*, and *N-ras* oncogenes. Further studies on the molecular mechanisms involved in the NIS pathophysiology may provide new information and result in more effective tools for the therapeutic management of thyroid carcinomas with impaired radioiodine uptake.

Restoration of NIS Gene Expression

It has been known for many years that TSH stimulates iodide transport into the thyroid gland via the adenylate cyclase-cAMP pathway. TSH has also been shown to increase NIS gene and protein expression in FRTL-5 cells and human thyrocyte monolayers, and this increase was accom-

panied by enhanced iodide transport activity (23). Forskolin and dibutyryl cAMP are able to mimic this stimulatory effect on iodide transport activity and on NIS gene and protein expression, suggesting that TSH regulates NIS expression through the cAMP signal transduction pathway (9).

Clinical pilot studies have shown that iodide uptake may be restimulated in patients with nonaccumulating thyroid tumors after treatment with retinoic acid (RA), a well-characterized agent with differentiation-inducing properties. Schmutzler et al. (24) found that iodide uptake and NIS mRNA were downregulated by RA in a cell culture model of normal, nontransformed thyrocytes, whereas NIS mRNA was upregulated in human follicular thyroid carcinoma cell lines. This differential response of NIS to RA may provide the possibility of upregulating iodide transport in thyroid carcinomas while simultaneously downregulating iodide accumulation in surrounding normal tissue. This would present a new perspective of tumor cell-targeted potential for radioiodine therapy.

Kogai et al. (14) reported that all-trans RA treatment stimulated NIS mRNA and protein in breast cancer cells. All-trans RA stimulated NIS gene transcription 4-fold and iodide uptake in a time- and dose-dependent fashion to up to 9.4-fold above baseline in MCF-7 estrogen receptor-positive breast cancer cells. Stimulation with selective retinoid compounds indicated that the induction of iodide uptake was mediated by RA receptor. No induction of iodide uptake was observed after RA treatment in an estrogen receptor-negative human breast cancer cell line, MDA-MB 231, or in a normal human breast cell line, MCF-12A. Stimulation of radioiodide uptake after systemic retinoid treatment may be useful for the diagnosis and treatment of some differentiated breast cancers.

A clinical study in 20 patients with advanced thyroid cancer (8 follicular, 7 papillary, and 5 oxyphilic) showed that 13-*cis*-RA treatment (1.5 mg/kg/d for 5 wk) was capable of reinducing iodine uptake in 50% of tumors (25). Simon et al. (26), reporting on the results of a multicenter clinical study, found that radioiodide uptake increased in 21 of 52 patients (40%). Moreover, in 4 (11%) of 36 patients, the tumor size regressed, and in 11 (31%) no further growth was detected. In another study on 12 patients, Grunwald et al. reported comparable results (27).

More recent results suggest that DNA methylation may be involved in the loss of functional NIS expression in thyroid cancer. Loss of radioiodide uptake in thyroid carcinomas could be due to reduced transcription caused by an aberrant methylation of the NIS gene.

Venkataraman et al. (28) found that in 7 human thyroid carcinoma cell lines lacking hNIS mRNA, treatment with 5-azacytidine or sodium butyrate was able to restore hNIS mRNA expression in 4 cell lines and iodide transport in 2 cell lines. An investigation of the methylation patterns in these cell lines revealed that the successful restoration of hNIS transcription was associated with the demethylation of hNIS DNA in the untranslated region within the first exon.

This was also associated with the restoration of the expression of thyroid transcription factor-1. These results suggest a role for DNA methylation in loss of hNIS expression in thyroid carcinomas and a potential application for chemical demethylation therapy in restoring responsiveness to therapeutic radioiodide. The methylation of thyroid-specific transcription factor genes causing loss of transcription factor expression with indirect loss of hNIS mRNA expression presents an alternative explanation for the loss of hNIS mRNA expression. The transcriptional downregulation of hNIS gene expression in thyroid carcinoma is presumed to be caused by the methylation of the DNA sequence in critical regulatory regions and to be reversed by chemical demethylation.

NIS EXPRESSION IN DISEASES

Autoimmune Thyroid Diseases

In Graves' disease, the majority of follicular cells were found to be strongly immunostained by NIS antibodies (4). Higher levels of NIS and TPO mRNAs were detected and were increased by 140- and 5-fold, respectively. In contrast, these tissues displayed normal levels of thyroglobulin, TSH receptor, and glucose transporter-1 (Glut-1) transcripts (29).

In autoimmune thyroiditis, follicular cells in close contact with lymphocytes were found to exhibit strong hNIS immunostaining, whereas cells distant from lymphocyte infiltrates were generally negatively or weakly immunostained. hNIS has recently been considered as a major autoantigen in autoimmune thyroiditis along with thyroglobulin, thyroperoxidase, and TSH receptor and, additionally, may attract cytotoxic lymphocytes. The heterogeneous hNIS distribution in these lesions can be related to tissue modifications due to lymphocytic infiltration (4).

All these immunostaining findings are consistent with thyroid scan findings in Graves' disease and Hashimoto's thyroiditis.

Thyroid Nodular Hyperplasia

In diffuse nodular hyperplasia, hNIS expression is heterogeneous and often confined to proliferating cells located at 1 pole of the follicle. Caillou et al. (4) found that there is a distinct zone of strongly positive proliferating follicular cells in the large follicles, whereas the nonproliferating cells were negative. Some macrofollicles displayed several areas of proliferating hNIS-positive cells. In normo- or microfollicular hyperplasia, hNIS immunostaining was similar to that found in normal thyroid tissue, but the number of positive cells was generally greater. This is in agreement with the data on the pathogenesis of nodular goiter, in which functional activity varies widely between follicles and even between individual cells within the same follicle.

In euthyroid hyperplastic glands, the mean expression of NIS mRNA was also found to be increased compared with that in normal thyroid tissues. However, the expressions of TPO, thyroglobulin, TSH receptor, and Glut-1 transcripts were normal (29).

Thyroid Adenoma

Caillou et al. (4) detected no hNIS immunostaining in the majority of follicles of cold adenomas. Only a few follicles were stained, and in these positive follicles, the number of positive cells was smaller than that in normal thyroid tissue. Lazar et al. (29) reported that NIS mRNA levels were decreased (2- to 700-fold lower) in the majority of samples studied, whereas the expressions of thyroglobulin, TSH receptor, and TPO genes were similar to that observed in normal thyroid tissue. However, higher levels of NIS were detected in toxic adenoma.

Thyroid Carcinoma

NIS Expression in Primary Tumor. Immunohistochemistry using NIS antibodies confirmed the much lower expression of NIS protein in thyroid cancer tissues and also demonstrated that its expression was heterogeneous, as it was detected in only a few malignant papillary or follicular thyroid cells (8).

In minimally invasive follicular carcinoma and in widely invasive or poorly differentiated follicular carcinomas, either a small minority of tumor cells was immunostained by the hNIS antibodies or immunostaining was absent. The number of positive cells was apparently higher in well-differentiated rather than in poorly differentiated cancer (4). In papillary carcinoma, hNIS immunostaining was negative or positive in only a few tumor cells. In the mixed papillary and follicular type, positivity was generally located in follicular structures, and in the follicular variant of papillary carcinoma, hNIS immunostaining was nonuniform. However, some tumor follicles proved to be positive in most tumor cells, though staining was heterogeneous and weaker than that in normal thyroid tissue. No NIS protein expression was detected in anaplastic or Hürthle cell carcinomas (9).

In neoplastic thyroid tissues, NIS mRNA expression was usually 10- to 1,200-fold lower than in normal tissues (median, 100-fold); TPO mRNA expression was reduced by 5- to 500-fold and thyroglobulin mRNA by 2- to 300-fold. Interestingly, the expression of TSH receptor transcripts was normal in most tumors. In addition, later tumor stages (stage > 1 as opposed to stage 1) were associated with lower levels of NIS ($P = 0.03$) and TPO ($P < 0.01$) expression (8).

However, Saito et al. (30) found by Northern blot analysis a 2.8-fold increase in the level of NIS mRNA in specimens with papillary carcinoma versus specimens with a normal thyroid. The NIS protein level was elevated in 7 of 17 cases of papillary carcinoma but was not elevated in the normal thyroid. Moreover, Dohan et al. (31) reported that as many as 70% of tumors exhibited increased NIS expression with respect to the normal surrounding thyroid tissue. These discordant results may be due to methodologic differences, particularly to the distinct peptide immunogens or to the procedure used for fixing the thyroid specimens. Most significantly, NIS was located in these samples either in both

the plasma membrane and intracellular compartments simultaneously or exclusively in intracellular compartments. This suggests that NIS is clearly expressed or even overexpressed in most thyroid cancer cells but that malignant transformation in some of these cells interferes either with the proper targeting of NIS to the plasma membrane or with the mechanisms that retain NIS in the plasma membrane after it has been targeted (31).

We found that NIS expression was related inversely to the degree of differentiation of thyroid cancer cells. NIS was always expressed in more differentiated thyroid cancer, whereas it was often negative in less well-differentiated thyroid cancers showing oxyphilic change and anaplastic transformation (32). Glut-1 expression was observed in NIS-negative specimens, and NIS was expressed in most Glut-1-negative specimens. Lazar et al. (29) also reported that the expression of the Glut-1 gene was increased 3- to 10-fold in NIS-negative cases. It is possible that NIS and Glut-1 expression reflect the biologic characteristics of cancer differentiation. The evaluation of NIS and Glut-1 expression might be helpful in determining the diagnostic and therapeutic modality, which is consistent with our experience that ^{18}F -FDG PET is useful for detecting metastatic lesions negative for ^{131}I on whole-body scanning (33).

One may conclude that hNIS expression may be either increased, decreased, or absent in well-differentiated thyroid cancer, even though the iodide transport activity is consistently decreased in the majority of tumors. Therefore, the explanation for the decreased iodide uptake in well-differentiated thyroid carcinomas does not lie simply in lower NIS expression but probably involves a more complex combination of regulatory changes ultimately affecting NIS expression, targeting, or activation. There clearly is a pressing need to investigate a much larger number of thyroid cancer specimens using more quantitative techniques to better understand the expression of hNIS both at the transcript and the protein levels, to elucidate the cellular localization of NIS, and to be able to compare such findings with the clinical behavior of the carcinoma (1).

NIS Expression in Metastatic Lesions. Another issue is whether NIS expression differs in metastases and primary cancer. Park et al. (34) investigated NIS mRNA levels in 7 pairs of primary and lymph node metastatic tissues. Despite NIS expression in the primary tumor, 2 of the 6 lymph node metastases did not express NIS mRNA, and levels of NIS mRNA in the remaining 4 lymph node metastases were lower than those in the primary tumors. One sample of lymph node metastatic tissue showed significant NIS mRNA expression, whereas no NIS mRNA was detected in the primary tumor. Arturi et al. (35) reported, after performing a dot blot analysis, that although NIS mRNA expression was maintained in thyroid tumor and in lymph node metastases, NIS expression was lower in metastatic tissue than in the respective primary cancer tissue.

Therefore, measurement of NIS expression levels in pri-

mary tissue might not predict the therapeutic response to ^{131}I in metastatic tissue. All published studies have involved primary cancers only, and thus the possibility exists that their metastases may display a different pattern of expression of thyroid-specific proteins. The possibility exists that a dedifferentiation process may occur in metastases; however, additional studies on this issue are necessary with respect to the clinical applications of such a finding (8).

Relation Between NIS Expression and ^{131}I Treatment Result. The evaluation of NIS expression might be useful for predicting the effectiveness of radioiodine therapy. Radioiodine whole-body scanning is useful in the selection of ^{131}I therapy for patients with recurrent or metastatic thyroid cancer. Although diagnostic radioiodine whole-body scanning is helpful, a significant false-negative rate has been reported compared with posttherapy ^{131}I scanning (36). In this respect, the evaluation of NIS expression has the potential to enhance patient management. In a limited number of cases, comparisons between iodide uptake on radioiodine scans and NIS expression patterns in thyroid carcinomas and metastases revealed positive correlations (9). We evaluated the outcome of ^{131}I therapy in 22 patients who showed ^{131}I uptake in recurrent lesions. Twelve patients responded to radioiodine therapy. Although only 4 of 12 patients (33.3%) with negative NIS immunostaining responded to radioiodine therapy, 8 of 10 patients (80%) with positive NIS immunostaining did so (37). Thyroid cancer tissues with NIS expression take up more ^{131}I than those without NIS expression and subsequently show a high rate of response to radioiodine therapy (Figs. 2 and 3). Therefore, we conclude that the determination of NIS expression levels might enhance patient management.

Breast Cancer and Stomach Cancer

In breast carcinoma, Tazebay et al. (17) found that 87% of the 23 invasive carcinomas and 83% of the 6 ductal carcinomas in situ expressed NIS, compared with only 23% of the 13 noncancerous samples adjacent to or in the vicinity of the tumors. In addition, none of the 8 normal breast samples expressed NIS. Both plasma membrane and intracellular staining were detected in some malignant breast cells, in contrast to the distinct basolateral plasma membrane staining of rat lactating mammary gland tissues and control salivary gland sections. Kilbane et al. (38) reported that NIS mRNA expression was demonstrated by reverse-transcription polymerase chain reaction in 6 of 7 breast carcinomas. NIS might be upregulated with a high frequency during malignant transformation in human breast cancer.

Wu et al. (39) reported a case of disseminated metastatic gastric adenocarcinoma with intensive radioiodine uptake. Shen et al. (16) showed that some metastatic bone lesions of gastric adenocarcinoma have NIS expression by reverse-transcription polymerase chain reaction. However, more accumulated data are needed to evaluate NIS expression in

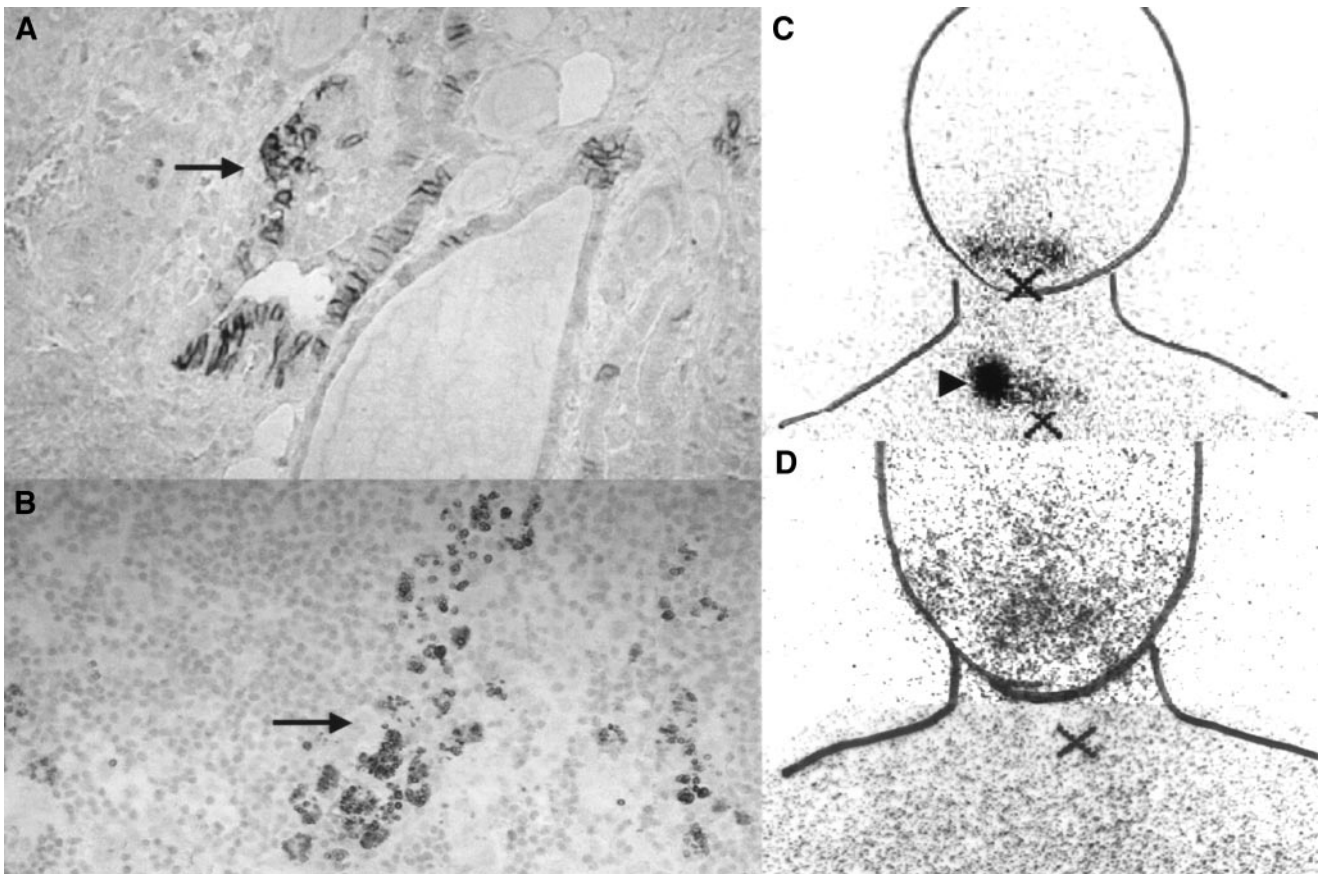


FIGURE 2. Immunohistochemical staining and ^{131}I whole-body scan findings of patient with papillary carcinoma. (A and B) In immunohistochemical staining ($\times 200$), cancer cells are immunostained for NIS (arrows). (C) Whole-body scan obtained 5 d after ingestion of 1.11 GBq ^{131}I shows functional metastatic lesions (arrowhead) in right cervical lymph nodes. (D) Six months after second radioiodine therapy (total administered dose, 11.1 GBq), radioactivity in right neck had completely disappeared. (Reprinted with permission of (37).)

gastric cancer. Nevertheless, if NIS expression in breast and gastric cancers can be increased sufficiently, some patients with breast and gastric cancers might benefit from $^{99\text{m}}\text{Tc}$ -pertechnetate diagnostic scanning or radioiodine therapy.

Congenital Hypothyroidism

The physiologic significance of NIS is best demonstrated in patients with an iodide transport defect (ITD), that is, in those with germline mutations in both NIS alleles. A congenital lack of iodide transport is a rather uncommon thyroid disorder and has been defined as an ITD. The general clinical picture consists of hypothyroidism, goiter, low thyroid radioiodine uptake, and low saliva-to-plasma iodide ratio. About 50 cases of ITD have been reported worldwide. Seventeen cases have been studied at the molecular level and shown to have a mutation in NIS (1). Perhaps because of its low incidence, little clinical interest has been shown in performing genetic testing for ITD among patients with congenital hypothyroidism. It is nonetheless important to identify ITD among children with congenital hypothyroidism so that their affected siblings, who may not yet be hypothyroid, can be identified (16).

AUTOANTIBODY AGAINST NIS

Recently, several lines of evidence have identified hNIS as a potential autoantigen in autoimmune thyroid diseases. Several authors have shown that antibodies in Graves' disease sera that can inhibit iodide uptake in CHO cells induce hNIS expression (38–42), which further emphasizes the role of NIS as a novel autoantigen in thyroid immunity.

Ajjan et al. (40) established a CHO cell line stably expressing hNIS but devoid of the last 31 amino acids, thus generating a valuable system (CHO-NIS cells) for the evaluation of anti-NIS antibodies. They found that 27 of 88 (30.7%) of the Graves' disease sera but none of the control sera inhibited iodide uptake. Kilbane et al. (38) found that sera from 30% of patients with Graves' disease had the ability to block radioactive iodide uptake by CHO-NIS cells and that this blocking activity was IgG related. Endo et al. (41) used recombinant rNIS protein and immunochemically searched for the anti-NIS autoantibody in the sera of patients with autoimmune thyroid disease. They found that 22 of 26 sera (85%) from patients with Graves' disease and 3 of 20 sera (15%) from patients with Hashimoto's thyroiditis

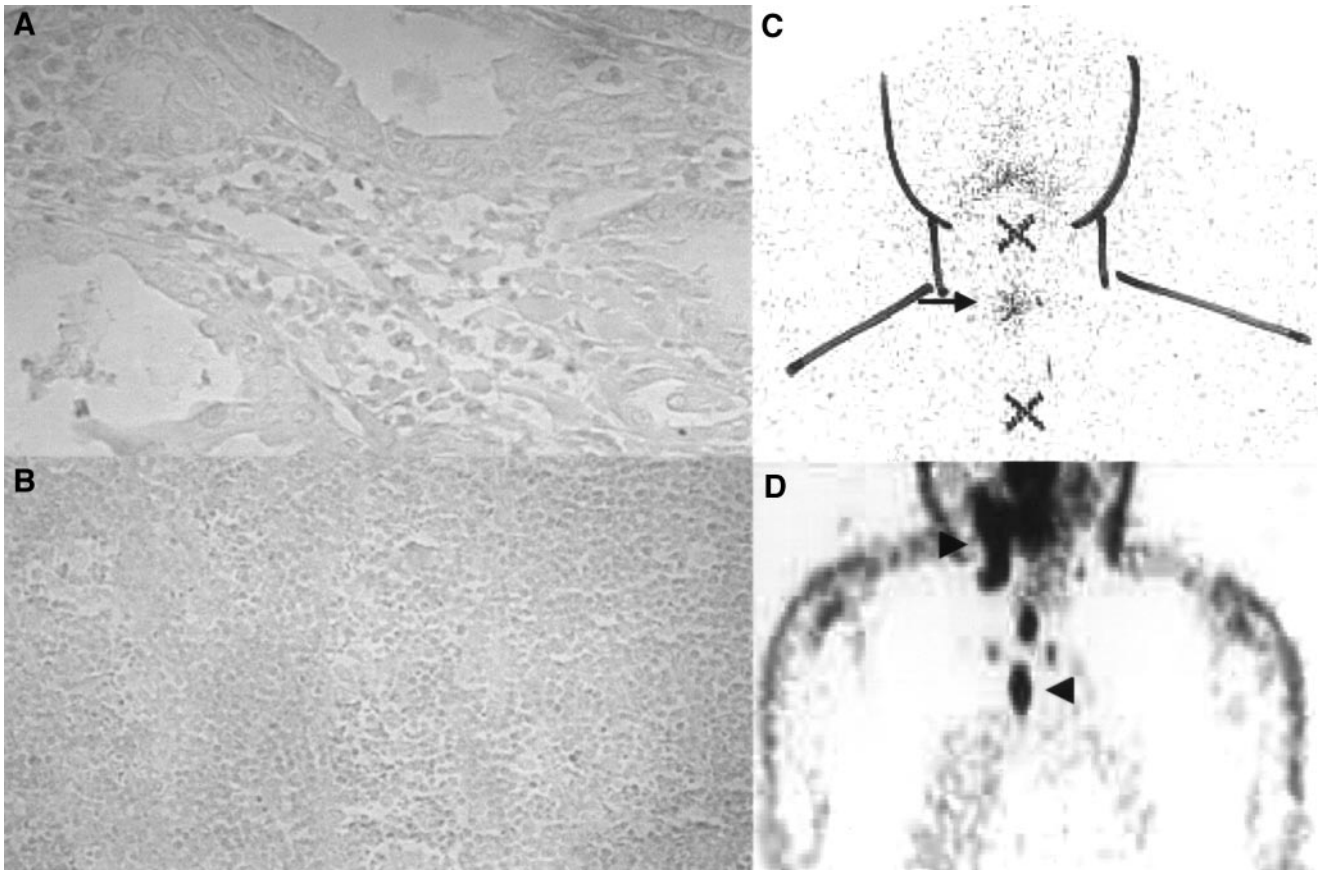


FIGURE 3. Immunohistochemical staining and ^{131}I whole-body scan findings of patient with papillary carcinoma. (A and B) In immunohistochemical staining for NIS ($\times 200$), NIS protein was not detected in paraffin block sections of either primary tumor (A) or lymph node (B). (C) Whole-body scan obtained 5 d after ingestion of 1.11 GBq ^{131}I shows no metastatic site; remnant thyroid activity is also shown (arrow). (D) ^{18}F -FDG PET scan obtained 3 wk later shows abnormally increased ^{18}F -FDG uptake on right side of neck and in mediastinum (arrowheads). (Reprinted with permission of (37).)

recognized anti-NIS autoantibody. No correlation was found between the staining intensity of NIS and TSH-binding inhibitor immunoglobulin activity or thyroid-stimulating antibody activity. They also found no relation between the intensity and the titer of anti-TPO antibody in the sera (39,42).

Although the function of this anti-NIS autoantibody remains to be elucidated, it seems possible that the autoantibody might affect iodide influx into thyrocytes and influence the thyroid function of patients with autoimmune thyroid disease. Autoantibodies to NIS could contribute to the pathogenesis of autoimmune thyroid disease by inhibiting one of the primary functions of the thyroid gland (15).

In addition, hNIS seems to be a potential autoantigen that may play a role in the pathogenesis of not only autoimmune thyroid disease but also associated autoimmune diseases. In various extrathyroidal tissues, such as gastric mucosa, salivary glands, and the lacrimal gland, hNIS may act as target antigen for T cells and cross-reacting autoantibodies and thus perhaps provides a link between autoimmune thyroid diseases and associated autoimmune diseases of other organ systems, such as autoimmune gastritis and Sjögren's syn-

drome. Given the expression of hNIS mRNA in various endocrine and secretory glands that are common targets in autoimmune diseases, NIS protein expression in these tissues and its potential role as a shared autoantigen in the evolution of polyglandular autoimmune syndromes warrants further attention (18).

NIS GENE FOR RADIONUCLIDE GENE THERAPY

NIS Gene Transfection to Thyroid Carcinoma

Iodine uptake is impaired in some cases of thyroid carcinoma. Approximately one third of differentiated thyroid cancers and all anaplastic thyroid cancers do not concentrate radioiodine. This prevents the use of radioiodine for the diagnosis and treatment of metastatic thyroid disease. The recent cloning and characterization of the hNIS gene have paved the way for the development of a novel cytoreductive gene therapy strategy for the treatment of thyroid cancer. The targeted expression of functional NIS in cancer cells would enable these cells to concentrate iodide from plasma and would therefore offer the possibility of radioiodine therapy (9).

Shimura et al. (43) transfected rNIS cDNA expression vector into malignantly transformed rat thyroid cells (FRTL-Tc), which did not concentrate iodide. The resultant cell line (Tc-rNIS) accumulated ^{125}I 60-fold in vitro. Tumors formed with Tc-rNIS cells accumulated up to 27.3% of total ^{125}I administered and concentrated the ^{125}I 11- to 27-fold in the tumors. Extracorporeal measurements of radioactivity in the tumors revealed that radioiodine accumulation peaked at 90 min and decreased to half of this level 6 h after injection. Smit et al. (44) reported the stable transfection of an hNIS expression vector into the hNIS-defective follicular thyroid carcinoma cell line FTC133. Stably transfected colonies exhibit high uptake of ^{125}I , which could be blocked completely with sodium perchlorate. Iodide uptake was maximal after 60 min, whereas iodide efflux was complete after 120 min. hNIS-transfected FTC133 and control cell lines injected subcutaneously into nude mice formed tumors after 6 wk. Iodide uptake in the hNIS-transfected tumors was much higher than in nontransfected tumors, and this corresponded to hNIS mRNA expression in tumors. We transfected the rNIS gene into a human anaplastic thyroid cancer cell line, ARO by liposome (ARO-N). Iodide uptake of ARO-N was 109 times higher than that of nontransfected cells. Iodide efflux (half-life) was 12 min in an in vitro experiment. In biodistribution studies using nude mice, ARO-N uptakes of ^{125}I , $^{99\text{m}}\text{Tc}$, and ^{188}Re were 18.5 ± 7.1 , 16.3 ± 6.7 , and 23.1 ± 3.5 %ID/g, respectively, at 2 h after injection (Fig. 4). The efflux (half-life) of ^{125}I , $^{99\text{m}}\text{Tc}$, and ^{188}Re in ARO-N tumors was 473, 756, and 131 min, respectively. These results demonstrate the possibility of radionuclide therapy in anaplastic thyroid carcinoma using NIS gene transfection (45).

This approach allowed the in vivo radioimaging of human tumor xenografted into mice. Moreover, in vitro experiments indicated that NIS-infected tumoral cells can be

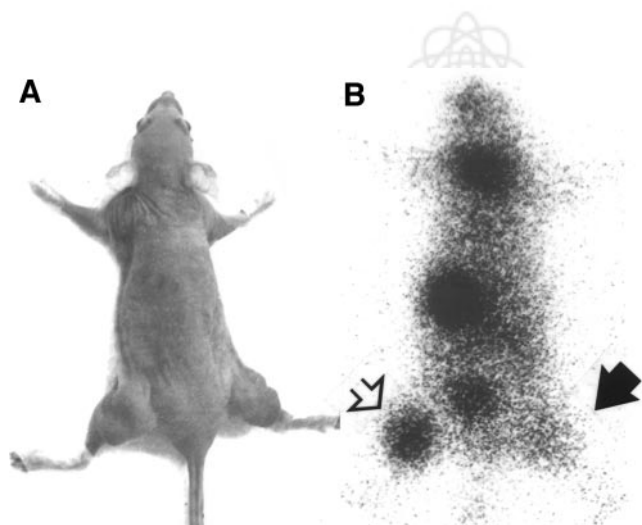


FIGURE 4. Whole-body photographic image (A) and scintigraphic image (B) of tumor-bearing nude mouse with subcutaneously transplanted wild-type human anaplastic thyroid cancer cells (ARO) (solid arrow) and rNIS-expressing cells (ARO-N) (open arrow) 30 min after injection of ^{131}I .

selectively killed by the induced accumulation of ^{131}I . The therapeutic efficacy of ^{131}I depends on the radiosensitivity of the tumor tissue and the biologic half-life of ^{131}I , which is dependent on the extent of radioiodine trapping and its retention time in the tumor. It is also possible to introduce the thyroperoxidase gene along with NIS to introduce iodide organification into targeted cells (9).

The possibility of achieving the expression and functional stimulation of the NIS gene in thyroid tumor cells has been exploited by physiologic (TSH) and pharmacologic agents (i.e., RA and protein kinase activator). Filetti et al. (8) suggest that transcriptional or posttranslational events, or both, and not a defective structural NIS gene, occur during the transformation process responsible for the low iodide uptake of thyrocytes. The elucidation of the other posttranscriptional and posttranslational events required to achieve the complete function of the thyroid iodide transport system, including the involvement of cofactors or other transporter molecules, such as the recently identified pendrin, will be necessary to determine the optimal conditions required to target thyroid tumor tissues with radioiodine.

NIS Gene Transfection to Nonthyroid Carcinoma

Combining the targeting and the expression of the NIS gene together with radioiodine treatment can also be used to treat nonthyroid malignant diseases. A novel cell line (MCF-3B) that stably expresses the NIS gene was established from MCF-7 breast cancer cells. This cell line took up 44 times more radioiodide in vitro than MCF-7. This iodide uptake was completely inhibited by perchlorate and was also dependent on external sodium and iodide concentrations. Iodide efflux from MCF-3B cells was slower (half-life, >27 min) than from FRTL-5 thyroid cells (half-life, 4 min). In a biodistribution study using MCF-3B-xenografted mice, high tumor uptake of ^{125}I was shown (16.73%) 1 h after injection, and ratios of tumor to normal tissue were also high (4.84–21.28). However, the iodide accumulation in the tumor reduced with time and was less than 1% at 24 h after injection (46).

Haberkorn et al. (47) reported that genetically modified MH3924A cell lines accumulated up to 235 times more iodide than noninfected hepatoma cells. Maximal iodide uptake in the cells was observed after 60 min of incubation. In rats, the hNIS-expressing tumors accumulated 6 times more iodide than did the contralateral wild-type tumor as monitored by scintigraphy. The ex vivo quantitation of iodide content performed 1 h after tracer administration in 1 g of tumor tissue revealed a 17-fold higher iodide accumulation in the genetically modified tumors. Mandell et al. (48) modified human melanoma, mouse colon carcinoma, and human ovarian adenocarcinoma cells with a retroviral vector bearing the rNIS gene and also observed up to a 35-fold increase in iodide uptake. Boland et al. (49) reported that the infection of SiHa cells (human cervix tumor cells) with adenovirus-NIS (AdNIS) resulted in ^{125}I uptake by these cells to a level 125–225 times higher than that of

noninfected cells. Similar results were obtained for other human tumor cell lines, including MCF-7 and T-47D (mammary gland), DU 145 and PC-3 (prostate), A549 (lung), and HT29 (colon), demonstrating that the AdNIS vector can function in tumor cells of various origins.

Boland (49) reported that quantitative analysis showed that uptake in AdNIS-injected tumors was 4–25 times higher than that in nontreated tumors. On average, 11% of the total amount of injected ^{125}I was recovered per gram of AdNIS-treated tumor tissue. Unfortunately, no difference in tumor size could be observed between groups of animals treated with AdNIS- ^{131}I and control groups treated with a control virus or with AdNIS alone (Table 1).

Many investigators have shown that gene transfer of NIS into a variety of cell types confers increased radioiodine uptake up to several hundredfold that of controls (16,48,50). In addition, some have demonstrated that NIS gene transfer mediated by nonviral or viral vectors into a variety of cells renders them susceptible to being killed by ^{131}I in vitro (48,49). Because NIS activity is the molecular basis of radioiodine therapy, there is great interest in exploring the possibility of NIS gene transfer to facilitate radioiodine therapy for nonthyroidal human cancers. However, to our knowledge, no definite therapeutic effect of ^{131}I on animals bearing tumors expressing exogenous NIS has been reported.

Targeted Gene Transfer

NIS gene transfer using tissue-specific promoters provides a way of selectively targeting the NIS gene to malignant cells, thereby maximizing tissue-specific cytotoxicity and minimizing toxic side effects in nonmalignant cells. One example is provided by prostate-specific promoter. After inducing tissue-specific iodide uptake activity by prostate-specific antigen promoter-directed NIS expression, the therapeutic effects of ^{131}I were demonstrated in prostate cancer in vitro and in vivo (51).

Spitzweg et al. (51) established the human prostate adenocarcinoma cell line LNCaP, which is stably transfected with NIS cDNA under the control of prostate-specific anti-

gen promoter. This prostate cancer cell line, NP-1, was found to be selectively killed by accumulated ^{131}I after inducing tissue-specific iodide uptake activity by prostate-specific antigen promoter-directed NIS expression in vitro. In an experiment on athymic nude mice, contrary to the NIS-negative control prostate tumors (P-1), which showed no in vivo uptake of ^{123}I , NP-1 tumors accumulated 25%–30% of the total ^{123}I administered, with a biologic half-life of 45 h. After a single intraperitoneal injection of a therapeutic ^{131}I dose (3 mCi), significant tumor reduction was achieved in NP-1 tumors in the therapy group versus P-1 tumors and tumors in the control group. Spitzweg et al. reported an average volume reduction of >90% and complete tumor regression in up to 60% of the tumors.

This kind of approach is possible after carefully selecting and modifying the promoter of the hNIS gene. Usually, the expression of the target gene is driven by relatively strong promoters, such as cytomegalovirus, which achieves a constitutive, high level of transcription. However, most tissue-specific promoters are weak in function. In applications involving a relatively weak, cellular promoter driving the expression of a gene, one has to contend with weaker transcriptional activity, which, in turn, limits the ability to accumulate a sufficiency of radionuclide in the cells. In such a case, it is essential to enhance the transcriptional activity of such a promoter. Several potential methods can be used: for example, chimeric promoters or 2-step transcriptional amplification (52).

Problems and the Future

Although the future of this approach appears to be promising for in vivo clinical applications, several questions remain unanswered and must be addressed. First, functional NIS targeting requires both the expression of the NIS gene and adequate posttranslational modification and trafficking of the NIS protein to the plasma membrane in target tumor cells, which are known to often lack differentiated functions. Gene expression can be efficiently monitored by engineering appropriate tissue-specific promoters or by controlling regulation (8). Assuming that the NIS gene is func-

TABLE 1
Previous Reports on Transfer of Sodium/Iodide Symporter Gene to Cancer Cells

Report	Cell line	Mode of transfer	Result	Therapeutic effect
Shimura et al. (43)	Rat thyroid malignant	Electroporation	60-fold	In vivo (–)
Smit et al. (44)	Human follicular thyroid cancer	Liposome	40-fold	NA
Lee et al. (45)	Human anaplastic thyroid cancer	Liposome	109-fold	NA
Nakamoto et al. (46)	Human breast cancer	Electroporation	44-fold	NA
Haberkorn et al. (47)	Human hepatoma	Retroviral vector	235-fold	NA
Mandell et al. (48)	Human melanoma	Retroviral vector	8- to 21-fold	In vitro (+)
Boland et al. (49)	Human cervix cancer	Adenoviral vector	125- to 225-fold	In vitro (+) In vivo (–)
Min et al. (50)	Human colon cancer	Liposome	10-fold	NA
Spitzweg et al. (51)	Human prostate cancer	Liposome with PSA promoter	60-fold	In vivo (+)

NA = not available.

tionally expressed at a sufficient level, the efficacy of its iodide-concentrating activity has to be demonstrated. Uptake, efflux, and saturation kinetics of iodide in tumor cells, particularly those of extrathyroidal origin, are likely to differ from those of normal thyroid cells. Moreover, extrathyroidal tissues do not efficiently accumulate and organify iodide, as does thyroid tissue, which results in a short iodide half-life. This suggests that the accumulation of radioactive iodide in tumor cells may not be adequate enough to achieve sufficient doses of radiation for therapeutic efficacy. Finally, there are also problems associated with the development of safe and efficient gene-delivery systems (8).

How can we maintain radioiodine in the cells? In normal thyroid tissue, the organification of iodide to thyroid hormone causes delayed excretion. The TPO gene is related to this excretory function in thyroidal cells, and TPO iodinated thyroglobulin tyrosine residues, resulting in iodide accumulation within thyrocytes. A way to overcome the rapid excretion of radioiodide from NIS-transfected tumors may be to transfer the TPO gene and thus induce organification (46). Huang et al. (53) showed that the transfection of non-small cell lung cancer cells with both hNIS and TPO genes resulted in an increase in radioiodide uptake and its retention and an enhanced rate of tumor cell apoptosis. The combination of NIS and TPO gene transfer, with resulting TPO-mediated organification and intracellular retention of radioiodide, may lead to a more effective means of inducing tumor cell death.

Another approach involves the use of the powerful β -emitting radionuclide, ^{188}Re , instead of ^{131}I . Rhenium is a chemical analog of technetium and has similar chemical properties. We found that ^{188}Re -perrhenate is accumulated in NIS-expressing cells and xenografted tumors as is ^{131}I or $^{99\text{m}}\text{Tc}$ pertechnetate (45). The emission characteristics and physical properties of ^{188}Re are superior to those of ^{131}I . It has a shorter physical half-life and is effective over a greater range, which is sufficient to eradicate medium- or large-sized tumors by the cross-fire effect. Dadachova et al. (54) performed dosimetry calculations by extrapolating mouse biodistribution data in humans and showed that ^{188}Re -perrhenate is able to deliver a dose 4.5 times higher than ^{131}I to an NIS-expressing breast tumor.

NIS GENE AS AN IMAGING REPORTER GENE

Assays for reporter gene expression are useful for evaluating molecular events. Commonly used reporter genes, such as β -galactosidase, green fluorescent protein, or luciferase, play critical roles in the development of gene delivery systems for gene therapy. However, measurement of the expression of these reporter genes often requires biopsy or death (55). It is necessary that techniques be developed to noninvasively and repetitively determine the location, duration, and magnitude of transgene expression in living animals. Because the radionuclide approach has sufficient sensitivity to quantitatively measure gene expression in

vivo, the approach of using imaging reporter genes with high-resolution PET scanning has been investigated (55). Accordingly, genes encoding for enzymes, receptors, antigens, and transporters have been used for this purpose.

Because expression of the HSV-tk gene leads to the phosphorylation of specific substrates of HSV-tk, such as ganciclovir, and to the accumulation of the resulting phosphorylated negatively charged metabolite, this gene has been used commonly as an *in vivo* reporter gene (56). The dopaminergic receptor gene (D_2R) and type II somatostatin receptor are considered to be possible imaging reporter genes because of their well-established imaging procedures. Other possible imaging reporter genes, such as the dopamine transporter and the glucose transporter-2, are of limited use because of the excessive size of their cDNAs (larger than 7 kb) (16).

Conventional imaging reporter genes, such as HSV1-tk and D_2R , require the synthesis of complicated substrates and expensive PET equipment. My group proposes that NIS may serve as an alternative imaging reporter gene, as its unique features compare favorably with those of HSV1-tk and D_2R (57). The feasibility of imaging NIS expression has been already demonstrated using ^{123}I or $^{99\text{m}}\text{Tc}$ -pertechnetate planar scintiscans. NIS has many advantages as an imaging reporter gene because of the wide availability of its substrates, that is, radioiodine and $^{99\text{m}}\text{Tc}$, and the well-understood metabolism and clearance of these substrates in the body. There is no problem of labeling stability when using radioiodine or $^{99\text{m}}\text{Tc}$ -pertechnetate, whereas this may be a major concern for the radiolabeled ligands of D_2R and type II somatostatin receptor (16). In addition, the NIS gene has another advantage, namely that it is not likely to interact with the underlying cell biochemistry. Iodide is not metabolized in most tissues, and although sodium influx may be a concern, no effects have been observed to date. The HSV-tk gene alters cellular behavior with respect to apoptosis by inducing changes in the deoxyribonucleoside triphosphate pool, and receptors may result in second-messenger activation. For the D_2R receptor system, a mutant gene has been applied and shows uncoupling of signal transduction (55).

Reporter gene imaging is an indirect approach toward visualizing the transcriptional and posttranscriptional regulation of target gene expression, as well as specific intracellular protein-protein interactions. Reporter gene imaging can also be used for monitoring cancer cells, immune cells, or neural stem cells. The hNIS gene can be transfected to specific cancer cells. Using animal models, the effect of anticancer therapeutic regimens can be monitored easily by a gamma camera system using radioiodine or $^{99\text{m}}\text{Tc}$ -pertechnetate. Immune cells with reporter gene can be used for monitoring and predicting anticancer effects, for evaluating graft-versus-host disease, and for investigating the pathogenesis of autoimmune disease. After the systemic injection of neural stem cells, stem cells and immortalized precursors may be able to migrate through the central nervous system

and repopulate ischemic sites. Using NIS as an imaging reporter gene, neural stem cell migration can be imaged (58).

CONCLUSION

After the discovery in the early 20th century that iodine specifically accumulates in the thyroid gland, the use of radioiodine has quickly adapted to clinical medicine and has led to the birth of nuclear medicine. Radioiodine is now widely used as a radioindicator for diagnostic studies such as thyroid uptake measurements and scintigraphy and as a radiopharmaceutical for therapy in patients with thyrotoxicosis or thyroid cancer. The mechanism for iodine specificity, however, has been elucidated only recently. The gene for the specific transporter, NIS, was identified in 1996. The cloning and characterization of the NIS gene has opened the way for the development of a novel gene therapy and radioiodine-based therapeutic strategy and a reporter gene imaging system. The NIS has the potential to expand the role of nuclear medicine in the future, just as it has served as the base for the development of nuclear medicine in the past.

REFERENCES

1. Vieja ADL, Dohan O, Levy O, Carrasco N. Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. *Physiol Rev.* 2000;80:1083–1105.
2. Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. *Nature.* 1996;379:458–460.
3. Daniels GH, Haber DA. Will radioiodine be useful in treatment of breast cancer? *Nat Med.* 2000;6:859–860.
4. 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.
5. Eskandari S, Loo DDF, Dai G, Levy O, Wright EM, Carrasco N. Thyroid Na⁺/I⁻ symporter. *J Biol Chem.* 1997;272:27230–27238.
6. Smanik PA, Liu Q, Furringer TL, et al. Cloning of the human sodium iodide symporter. *Biochem Biophys Res Commun.* 1996;226:339–345.
7. Levy O, De la Vieja A, Carrasco N. The Na⁺/I⁻ symporter (NIS): recent advances. *J Bioenerg Biomembr.* 1998;30:195–206.
8. Filetti S, Bidart J-M, Arturi F, Caillou B, Russo D, Schlumberger M. Sodium/iodide symporter: a key transport system in thyroid cancer cell metabolism. *Eur J Endocrinol.* 1999;141:443–457.
9. Spitzweg C, Harrington KJ, Pinke LA, Vile RG, Morris JC. The sodium/iodide symporter and its potential role in cancer therapy. *J Clin Endocrinol Metab.* 2001;86:3327–3335.
10. Levy O, Dai G, Riedel C, et al. Characterization of the thyroid Na⁺/I⁻ symporter with an anti-COOH terminus antibody. *Proc Natl Acad Sci USA.* 1997;94:5568–5573.
11. Uyttensproot N, Pelgrims N, Carrasco N, et al. Moderate doses of iodide in vivo inhibit cell proliferation and expression of thyroid peroxidase and the Na⁺/I⁻ symporter mRNAs in dog thyroid. *Mol Cell Endocrinol.* 1997;131:195–203.
12. Eng PH, Cardona GR, Fang SL, et al. Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein. *Endocrinology.* 1999;140:3401–3410.
13. Ajjan RA, Kamaruddin NA, Crisp M, Watson PF, Ludgate M, Weetman AP. Regulation and tissue distribution of the human sodium iodide symporter gene. *Clin Endocrinol.* 1998;49:517–523.
14. Kogai T, Schultz JJ, Johnson LS, Huang M, Brent GA. Retinoic acid induces sodium/iodide symporter gene expression and radioiodide uptake in the MCF-7 breast cancer cell line. *Proc Natl Acad Sci USA.* 2000;97:8519–8524.
15. Ohmori M, Endo T, Harii N, Onaya T. A novel thyroid transcription factor is essential for thyrotropin-induced up-regulation of Na⁺/I⁻ symporter gene expression. *Mol Endocrinol.* 1998;12:727–736.
16. Shen DHY, Kloos RT, Mazafferri EL, Jhiang SM. Sodium iodide symporter in health and disease. *Thyroid.* 2001;11:415–425.
17. Tazebay UH, Wapnir IL, Levy O, et al. The mammary gland iodide transporter is expressed during lactation and in breast cancer. *Nat Med.* 2000;6:871–878.
18. Spitzweg C, Joba W, Eisenmenger W, Heufelder AE. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland, and gastric mucosa. *J Clin Endocrinol Metab.* 1998;83:1746–1751.
19. Trapasso F, Iuliano R, Chiefari E, et al. Iodide symporter gene expression in normal and transformed rat thyroid cells. *Eur J Endocrinol.* 1999;140:447–451.
20. Kogai T, Hershman JM, Motomura K, Endo T, Onaya T, Brent GA. Differential regulation of the human sodium/iodide symporter gene promoter in papillary thyroid carcinoma cell lines and normal thyroid cells. *Endocrinology.* 2001;142:3369–3379.
21. Magliano MP, Lauro RD, Zannini M. Pax8 has a key role in thyroid cell differentiation. *Proc Natl Acad Sci USA.* 2000;97:13144–13149.
22. Russo D, Manole D, Arturi F, et al. Absence of sodium/iodide symporter gene mutations in differentiated human thyroid carcinomas. *Thyroid.* 2001;11:37–39.
23. Spitzweg C, Heufelder AE. The sodium iodide symporter: its emerging relevance to clinical thyroidology. *Eur J Endocrinol.* 1998;138:374–375.
24. Schmutzler C, Winzer R, Meissner-Weigl J, Koehrl 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.
25. Simon D, Koehrl J, Reiners C, et al. Redifferentiation therapy with retinoids: therapeutic option for advanced follicular and papillary carcinoma. *World J Surg.* 1998;22:569–574.
26. Simon D, Koehrl J, Reiners C, et al. Redifferentiation therapy of thyroid cancer: results of a multicenter pilot study [abstract]. *Thyroid.* 1998;8:1217.
27. Grunwald F, Menzel C, Bender H, et al. Redifferentiation therapy-induced radioiodine uptake in thyroid cancer. *J Nucl Med.* 1998;39:1903–1906.
28. 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.
29. Lazar V, Bidart J-M, 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.
30. Saito T, Endo T, Kawaguchi A, et al. Increased expression of the sodium/iodide symporter in papillary thyroid carcinomas. *J Clin Invest.* 1998;101:1296–1300.
31. Dohan O, Baloch Z, Banreji Z, Livolsi V, Carrasco N. Predominant intracellular overexpression of the Na⁺/I⁻ symporter (NIS) in a large sampling of thyroid cancer cases. *J Clin Endocrinol Metab.* 2001;86:2697–2700.
32. Min J-J, Chung J-K, Lee YJ, et al. Relationship between differentiation and expression of sodium/iodide symporter or glucose transporter-1 in differentiated thyroid carcinoma [abstract]. *J Nucl Med.* 2001;42(suppl):133P.
33. Chung J-K, So Y, Lee JS, et al. Value of FDG PET in papillary thyroid carcinoma with negative ¹³¹I whole-body scan. *J Nucl Med.* 1999;40:986–992.
34. Park HJ, Kim JY, Park KY, Gong G, Hong SJ, Ahn IM. Expressions of human sodium iodide symporter mRNA in primary and metastatic papillary thyroid carcinoma. *Thyroid.* 2000;10:211–217.
35. Arturi F, Russo D, Schlumberger M, et al. Iodide symporter gene expression in human thyroid tumors. *J Clin Endocrinol Metab.* 1998;83:2493–2496.
36. Lee BW, Lee DS, Moon DH, et al. Comparison of I-131 diagnostic scan and therapeutic scan in thyroid carcinoma. *Korean J Nucl Med.* 1990;24:80–86.
37. Min J-J, Chung J-K, Lee YJ, et al. Relationship between expression of the sodium/iodide symporter and ¹³¹I uptake in recurrent lesions of differentiated thyroid carcinoma. *Eur J Nucl Med.* 2001;28:639–645.
38. Kilbane MT, Ajjan RA, Weetman AP, et al. Tissue iodine content and serum-mediated ¹²⁵I uptake-blocking activity in breast cancer. *J Clin Endocrinol Metab.* 2000;85:1245–1250.
39. Wu SY, Kollin J, Coodley E, et al. I-131 total-body scan: localization of disseminated gastric adenocarcinoma. *J Nucl Med.* 1984;25:1204–1209.
40. Ajjan RA, Findlay C, Metcalfe RA, et al. The modulation of the human sodium iodide symporter activity by Graves' disease sera. *J Clin Endocrinol Metab.* 1998;83:1217–1221.
41. Endo T, Kogai T, Nakazato M, Saito T, Kaneshige M, Onaya T. Autoantibody against Na⁺/I⁻ symporter in the sera of patients with autoimmune thyroid disease. *Biochem Biophys Res Commun.* 1996;224:92–95.
42. Seissler J, Wagner S, Schott M, et al. Low frequency of autoantibodies to the human Na⁺/I⁻ symporter in patients with autoimmune thyroid disease. *J Clin Endocrinol Metab.* 2000;85:4630–4634.
43. Shimura H, Haraguchi K, Miyazaki A, Endo T, Onaya T. Iodide uptake and experimental ¹³¹I therapy in transplanted undifferentiated thyroid cancer cells expressing the Na⁺/I⁻ symporter gene. *Endocrinology.* 1997;138:4493–4496.
44. Smit JWA, Elst JPS, Karperien M, et al. Reestablishment of in vitro and in vivo

- iodide uptake by transfection of the human sodium iodide symporter (hNIS) in a hNIS defective human thyroid carcinoma cell line. *Thyroid*. 2000;10:939–943.
45. Lee Y, Chung J, Shin J, et al. Animal model of a human anaplastic thyroid cancer cell line, ARO-N, expressing sodium/iodide symporter [abstract]. *J Nucl Med*. 2002;43(suppl):119P.
 46. Nakamoto Y, Saga T, Misaki T, et al. Establishment and characterization of a breast cancer cell line expressing Na⁺/I⁻ symporters for radioiodide concentrator gene therapy. *J Nucl Med*. 2000;41:1898–1904.
 47. Haberkorn U, Henze M, Altmann A, et al. Transfer of the human NaI symporter gene enhances iodide uptake in hepatoma cells. *J Nucl Med*. 2001;42:317–325.
 48. Mandell RB, Mandell LZ, Link CJ Jr. Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. *Cancer Res*. 1999;59:661–668.
 49. Boland A, Ricard M, Opolon P, et al. Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for a targeted radiotherapy. *Cancer Res*. 2001;60:3484–3492.
 50. Min JJ, Chung J-K, Lee YJ, et al. In vitro and in vivo characteristics of a human colon cancer cell line, SNU-C5N, expressing sodium/iodide symporter. *Nucl Med Biol*. 2002;in press.
 51. Spitzweg C, O'Connor MK, Bergert ER, et al. Treatment of prostate cancer by radioiodine therapy after tissue-specific expression of the sodium iodide symporter. *Cancer Res*. 2000;60:6526–6530.
 52. Iyer M, Carey M, Wang Y, Smallwood A, Gambhir SS. Two-step transcriptional amplification as a method for imaging reporter gene expression using weak promoters. *Proc Natl Acad Sci USA*. 2001;98:14595–14600.
 53. Huang M, Batra RK, Kogai T, et al. Ectopic expression of the thyroperoxidase gene augment radioiodide uptake and retention mediated by the sodium iodide symporter in non-small cell lung cancer. *Cancer Gene Ther*. 2001;8:612–618.
 54. Dadachova E, Bouzahzah B, Zuckier LS, Pestell RG. Rhenium-188 as an alternative to iodine-131 for treatment of breast tumors expressing the sodium/iodide symporter (NIS). *Nucl Med Biol*. 2002;29:13–18.
 55. Haberkorn U, Altmann A, Eisenhut M. Functional genomics and proteomics: the role of nuclear medicine. *Eur J Nucl Med*. 2002;29:115–132.
 56. 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 USA*. 1999;96:2333–2338.
 57. Shin JH, Chung J-K, Lee YJ, et al. The possibility of sodium/iodide symporter (NIS) gene as a new imaging reporter gene system [abstract]. *J Nucl Med*. 2002;43(suppl):271P.
 58. Shin JH, Chung J-K, Roh JK, et al. Monitoring of neural stem cell using sodium/iodide symporter gene [abstract]. *J Nucl Med*. 2002;43(suppl):238P.

