The Sodium Iodide Symporter and Its Potential Role in Cancer Therapy

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Active transport of iodide into the thyroid gland is a crucial and rate-limiting step in the biosynthesis of thyroid hormones that play an important role in the metabolism, growth, and maturation of a variety of organ systems, in particular the nervous system (1). Although it has been known for decades that iodide transport into the thyroid gland is mediated by a specific sodium-dependent iodide transporter located at the basolateral membrane of thyroid follicular cells, the sodium iodide symporter (NIS) gene was cloned just 4 yr ago (2, 3). After cloning of the rat sodium iodide symporter (rNIS) from a Fisher rat thyroid line (FRTL-5)-derived complementary DNA (cDNA) library (2), the human sodium iodide symporter (hNIS) was cloned from a human thyroid cDNA library in 1996 (3). The hNIS gene is localized on chromosome 19p12–13.2 and encodes a glycoprotein of 70–90 kDa. The coding region of hNIS contains 15 exons interrupted by 14 introns and codes for a 3.9-kb messenger ribonucleic acid (mRNA) transcript (4). 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 three potential N-linked glycosylation sites; 1 is located in the fourth extracellular (seventh extramembranous domain), and 2 are located in the last extracellular (13th extramembranous domain) loop (5) (Fig. 1).

NIS cotransports two sodium ions along with one iodide ion, with the transmembrane sodium gradient serving as the driving force for iodide uptake. The sodium gradient providing the energy for this transfer is generated by the ouabain-sensitive Na+/K+-adenosine triphosphatase (Na+/K+-ATPase). NIS-mediated iodide transport is, therefore, inhibited by the Na+/K+-ATPase inhibitor ouabain as well as by the competitive inhibitors thiocyanate and perchlorate (1) (Fig. 2). After active transport across the basolateral membrane of thyroid follicular cells, iodide is translocated across the apical membrane by pendrin, the Pendred syndrome gene product, which is a chloride/iodide transporter (6–10) (Fig. 2). Other apical anion transporters may also be involved. At the cell/colloid interface iodide is organified in a complex reaction involving oxidation catalyzed by thyroid peroxidase (TPO) and incorporation into tyrosyl residues along the thyroglobulin (Tg) backbone. The thyroid hormones T3 and T4 are synthesized by coupling of two iodotyrosine residues and are stored in the colloid (Fig. 2). The iodide organification step can be inhibited by propylthiouracil and methimazole, which are TPO enzyme inhibitors. All of these steps are stimulated through pituitary-derived TSH, which interacts with the TSH receptor at the basolateral membrane of thyroidal cells (1). It has been known for many years that TSH stimulates iodide transport into the thyroid gland via the adenylate cyclase-cAMP pathway (1). After NIS was cloned, several studies in FRTL-5 cells and cultured human thyroid cells showed that treatment with TSH stimulates iodide transport activity as well as NIS gene and protein expression (11, 12). Forskolin and dibutyryl cAMP are able to mimic this stimulatory effect on both iodide transport activity as well as NIS gene and protein expression, suggesting that TSH regulates NIS expression through the cAMP signal transduction pathway (11) (Fig. 2).

In addition to its key role in thyroid physiology, NIS-mediated iodide accumulation in the thyroid gland is a crucial prerequisite for diagnostic scintigraphic imaging as well as for the highly efficient radioiodine therapy of benign and malignant thyroid diseases. The purpose of this review is to summarize and discuss the current knowledge of NIS and its diagnostic and therapeutic implications in thyroidal and nonthyroidal cancer.

Radioiodine therapy of thyroid cancer based on thyroidal NIS expression

The unique property of thyroid follicular cells to trap and concentrate iodide due to expression of NIS allows imaging as well as effective therapy of differentiated thyroid carcinomas and their metastases by administration of radioiodine, thereby improving the prognosis of thyroid cancer patients significantly and making thyroid cancer one of the most manageable cancers (13). Differentiated thyroid carci-
nomas, including papillary (75–85%) and follicular (10–20%) carcinomas, are usually treated by total or near-total thyroidectomy followed by $^{131}$I ablation of the thyroid remnant and occult microscopic carcinomas. Subsequent postablative $^{131}$I total body scanning can diagnose local and metastatic residual and recurrent disease. Therapy with $^{131}$I has been successfully used for over 40 yr in the treatment of differentiated thyroid cancer. Recurrence rates are significantly higher in patients treated with surgery and TSH suppression by $T_4$ alone than in those who also receive radioiodine treat-

FIG. 1. Schematic model of the hNIS, which represents an intrinsic membrane protein with 13 transmembrane and 14 extramembranous (ExM) domains and 3 potential N-linked glycosylation sites.

FIG. 2. Schematic illustration of the key aspects of iodine transport and organification in the thyroid gland. TSHR, TSH receptor; MMI, methimazole; PTU, propylthiouracil.
The efficacy of radiiodine therapy is reflected in the low mortality of patients suffering from metastatic thyroid cancer who are treated with $^{131}$I (3%) compared with those who are not (12%). Even young patients with diffuse pulmonary metastases at initial presentation can be successfully treated with $^{131}$I, achieving a 10-yr survival of over 80% (13).

Thyroidal NIS expression therefore opens the door to effective cancer therapy, which is remarkably free of serious adverse effects, except for transient and usually mild salivary ductitis due to NIS expression in the salivary glands and reversible myelosuppression (13, 14). The effect of $^{131}$I is related to the effective radiation dose delivered to the tumor tissue, which depends on the effective half-life of $^{131}$I in the tumor and the tumor's capacity to concentrate $^{131}$I. The prognosis of metastatic thyroid cancer is much worse when the local and metastatic disease does not concentrate $^{131}$I in amounts sufficient for a therapeutic effect, which is a more common situation in patients over the age of 40 yr.

Cloning of the NIS gene in 1996 (2, 3) has facilitated investigation of the molecular mechanisms underlying decreased levels of radiiodine accumulation in thyroid cancer tissues and their metastases that limit the therapeutic efficacy of $^{131}$I. Smanik et al. reported a much lower level of NIS mRNA expression in thyroid carcinoma tissues (2 papillary, 1 follicular, and 1 anaplastic) compared with that in normal thyroid tissue using Northern blot analysis. Using RT-PCR the same researchers found variable levels of NIS expression in a panel of different papillary carcinoma tissues, which is consistent with the clinical observation of variable response of papillary carcinoma to radiiodine treatment (4). Further, using RT-PCR no NIS mRNA expression was detected in 5 human thyroid carcinoma cell lines that have lost iodide uptake activity (3). In another series of thyroid carcinomas (19 papillary, 5 follicular, and 2 anaplastic), Arturi et al. used RT-PCR to detect loss of NIS mRNA expression in 5 of 19 papillary cancers, 1 of 5 follicular cancers, and both anaplastic cancers (15). In a more recent study using a kinetic quantitative RT-PCR method, NIS mRNA expression was shown to be decreased in 40 of 43 thyroid carcinomas (38 papillary and 5 follicular) and in 20 of 24 cold adenomas compared with normal thyroid tissue, whereas NIS mRNA levels were increased in each of 8 toxic adenomas and 5 Graves' thyroid tissues. In thyroid cancer tissues, a positive correlation was found among the expression levels of NIS, TPO, Tg, and TSH receptor, and higher tumor stages were associated with lower levels of NIS expression (16). Park et al. investigated NIS mRNA levels in 23 papillary carcinomas and 7 pairs of primary and lymph node metastatic tissues by RT-PCR and ribonuclease protection assay. Three of 23 papillary carcinomas did not express NIS mRNA, and the rest showed variable levels of NIS mRNA expression that were lower than those in normal thyroid tissue. Despite NIS expression in the primary tumor, 2 of 6 lymph node metastases did not express NIS mRNA. Levels of NIS mRNA expression 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. Therefore, as no correlation was found between NIS expression levels in primary and lymph node metastatic tissue, measurement of NIS expression levels in primary tissue cannot predict the therapeutic response to $^{131}$I in the metastatic tissue (17). Further, NIS mRNA expression levels have been shown to be decreased in oncogene-transformed PC 3 rat thyroid cell lines (PC v-erbB, PC HaMSV, PC v-raf, and PC E1A) and were almost completely absent in PC RET/PTC, PC KiMSV, PC p53 (143 Ala), and PC PyMLV. These data suggest that oncogene activation may be involved in the pathophysiology of reduced NIS expression in thyroid cancer (18).

The availability of specific polyclonal and monoclonal anti-hNIS antibodies has allowed investigation of NIS protein expression levels in various malignant thyroid tissues. In contrast to normal thyroid tissue, which reveals heterogeneous NIS protein expression at the basolateral membrane of a minority of follicular cells (19, 20), most studies showed decreased levels of NIS protein expression with less pronounced basolateral orientation in malignant thyroid tumors (19–22). Using a rabbit polyclonal hNIS-specific antibody (aa 615–643), immunohistochemical analysis of five follicular and nine papillary carcinomas revealed low or absent NIS protein expression, with NIS protein expression levels correlating with differentiation levels (20). Interestingly, the number of TSH receptor-positive cells was also decreased in thyroid carcinomas, which may at least in part explain the decreased levels of NIS expression and iodide-concentrating capacity in thyroid carcinomas, given that TSH is an important stimulator of NIS gene and protein expression. In a limited number of cases, comparison of iodide uptake on radiiodine scans and NIS expression patterns in thyroid carcinomas and metastases revealed a positive correlation, suggesting that NIS expression levels might predict the therapeutic efficacy of radiiodine therapy in thyroid cancer (20).

However, this concept requires confirmation in a larger series of thyroid cancer patients. Consistent with these findings, Jhiang et al. reported no NIS protein expression in three papillary carcinomas and one follicular carcinoma using a rabbit polyclonal hNIS-specific antibody (aa 468–643) (22). No NIS protein expression was detected in anaplastic and Hurthle cell carcinomas (19, 21).

The studies summarized above suggest that a reduction in NIS expression may account at least in part for the reduced iodide uptake activity generally observed in thyroid cancer tissue. In contrast to these data, Northern blot and immunoblot analysis as well as immunohistochemical staining of 31 papillary carcinomas by Saito et al. using a rabbit polyclonal hNIS-specific antibody (aa 466–522) showed elevated NIS expression levels in approximately 50% of the tumors. The researchers attempted to explain the decreased levels of radiiodine accumulation despite elevated NIS expression levels by NIS inactivation, possibly due to NIS gene mutations, different posttranslational modifications such as glycosylation, or decreased TSH responsiveness (23).

Therefore, although most data available to date suggest that decreased NIS expression levels account for the reduced iodide uptake activity in thyroid carcinomas, this and other possible mechanisms, including alterations in NIS gene structure, NIS gene and protein regulation, posttranslational modification, NIS protein synthesis, and cellular localization
of NIS, have not been investigated or confirmed in a larger series of thyroid cancer specimens.

The pivotal role of NIS in the responsiveness of thyroid cancer to $^{131}$I has led investigators to examine the possibility of designing therapies to enhance functional NIS expression in dedifferentiated thyroid cancers, thus restoring their susceptibility to radioiodine treatment.

Retinoic acid (RA), a well characterized reagent with differentiation-inducing properties, has been shown to suppress iodide uptake and NIS mRNA levels in normal, non-transformed FRTL-5 cells, whereas NIS mRNA expression levels were up-regulated in human follicular thyroid carcinoma cell lines in vitro (24). 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/day for 5 weeks) was capable of reinducing iodine uptake in 50% of tumors (25). Treatment with RA may therefore provide a means of reestablishing the therapeutic efficacy of radioiodine therapy by targeted up-regulation of iodide transport in thyroid cancer cells while down-regulating iodide accumulation in surrounding normal thyroid tissue.

More recent data suggest that DNA methylation may be involved in the loss of functional NIS expression in thyroid cancer. Analysis of NIS mRNA expression in 23 thyroid cancer samples showed correlation of the loss of NIS expression with the loss of clinical radioiodine uptake. However, some of the thyroid carcinoma samples with NIS expression did not concentrate iodide, suggesting additional posttranscriptional mechanisms for loss of NIS function. In 7 human thyroid carcinoma cell lines without NIS mRNA expression, demethylation treatment with 5-azacytidine or sodium butyrate restored NIS mRNA expression in 4 cell lines and iodide uptake in 2 cell lines. Investigation of methylation patterns in these cell lines revealed that successful restoration of NIS transcription was associated with demethylation of NIS DNA in the untranslated region within the first exon. These data suggest a role for DNA methylation in the loss of NIS expression and function in thyroid carcinomas as well as a potential application for chemical demethylation therapy in restoring responsiveness to $^{131}$I (26).

In conclusion, most studies suggest that a decrease in or loss of NIS expression may play a central role in the defective iodide concentration capacity of thyroid carcinomas. Further investigation of the exact underlying molecular mechanisms may provide an important tool for the development of new diagnostic and therapeutic strategies for improved thyroid cancer management.

**NIS expression in mammary gland and its possible implications in treatment of breast cancer**

It has been known for decades that in addition to thyroidal iodide transport, active iodide accumulation occurs in a variety of nonthyroidal tissues, including salivary glands, gastric mucosa, and lactating mammary glands (1). Iodide transport in these nonthyroidal tissues is TSH independent, but reveals several functional similarities to that in the thyroid gland, such as inhibition by thiocyanate and perchlorate and generation of iodide concentration gradients of similar magnitude (1). In addition, numerous data suggest that iodide is organified by certain extrathyroidal tissues (27, 28).

As thyroidal NIS was cloned, NIS mRNA and protein expression has been reported in a variety of extrathyroidal tissues, including salivary and lacrimal glands, gastric mucosa, kidney, and mammary gland, suggesting that iodide transport in these tissues is mediated by the expression of functional NIS protein (22, 29–32). Iodide accumulation in breast tissue was first reported over 40 yr ago (33). As with organification of iodide in the thyroid gland, about 20% of the trapped iodide has been shown to be organified in lactating mammary gland as a result of iodide oxidation by peroxidase expressed in the alveolar cells of the breast followed by binding to tyrosyl residues of caseins and other milk proteins (27, 34). In the lactating mammary gland iodide is actively transported and secreted into the milk, thereby supplying iodide to the infant for the biosynthesis of thyroid

![Fig. 3. Mammary gland NIS and its physiological and potential diagnostic and therapeutic implications.](image-url)
hormones that are essential for the development of the nervous system, skeletal muscle, and lung (1) (Fig. 3).

In addition, a role for iodine in the prevention of breast dysplasia and hyperplasia has been suggested (35–37). In a recent study, Kilbane et al. demonstrated that the tissue iodide content of breast carcinomas was significantly lower than that in remote normal tissue from the tumor-bearing breast or in fibroadenomata. It has therefore been proposed that a disorder of iodide uptake may be involved in the development of breast cancer, which might be due to NIS-inhibiting antibodies that are present in 19.6% of sera from breast carcinoma patients (38) (Fig. 3).

Very recently, the group led by N. Carrasco identified the mammary gland NIS protein (mgNIS) and demonstrated that it mediates iodide accumulation in lactating mammary glands (32). By Western blot analysis using a high affinity antibody directed against the carboxyl-terminus of rat NIS, mgNIS was detected as a single band of approximately 75 kDa in mammary gland membranes of lactating rats. The same antibody detected NIS as a single band of about 100 kDa in rat thyroid gland, suggesting that rat mgNIS reveals a lesser degree of glycosylation than thyroidal rat NIS. In both cases deglycosylated NIS protein was detected as a band of about 50 kDa. Immunohistochemical analysis of paraffin-embedded tissue sections derived from lactating mammary gland showed distinct mgNIS-specific immunoreactivity at the basolateral membrane of alveolar epithelial cells (32). Examination of mgNIS protein expression at various physiological stages showed that mgNIS is exclusively present in the mammary gland during gestation and lactation, in contrast to the constitutive expression of NIS in the thyroid gland. mgNIS was absent in nubile mammary glands, but was present in lactating mammary glands. Twenty-four hours after mice were weaned, mgNIS protein expression was substantially decreased and was undetectable by 48 h. Reinitiation of suckling reestablished mgNIS expression (32). These data suggest that hormones involved in active lactation stimulate mgNIS expression and/or its functional activity. Hormonal regulation studies in intact and ovariectomized mice showed rather complicated regulation mechanisms for mgNIS by estrogen, PRL, and oxytocin. A threshold level of circulating estrogens seemed to be necessary for optimal mgNIS expression in lactating mammary gland tissue, in particular for the up-regulation of mgNIS by oxytocin. A combination of estrogens, PRL and oxytocin, which resembles the relative hormonal levels in mice during lactation, led to the highest levels of mgNIS expression in ovariectomized mice (32). In accordance with these findings, Cho et al. showed that both NIS protein expression levels and radioiodine uptake in rat mammary gland are maximal during active lactation compared with those in mammary glands of virgin and pregnant rats and in the involuting mammary gland. Radioiodine uptake into lactating mammary glands was partially inhibited by treatment with a selective oxytocin antagonist or bromocriptine, an inhibitor of PRL release, suggesting that radioiodine uptake in the mammary gland is at least in part modulated by oxytocin and PRL. In addition, using real-time quantitative RT-PCR NIS mRNA levels have been shown to be increased in a dose-dependent manner by oxytocin and PRL in three-dimensional histocultured human breast tumors (39). Furthermore, PRL stimulation of iodide uptake by cultured mouse mammary tissues taken from pregnant mice had already been reported even before NIS was cloned and has recently been shown to be due to increased NIS protein expression (40–42). Further in vitro and in vivo regulation studies are still needed to understand fully the hormonal regulation mechanisms of NIS expression and function in benign and malignant breast tissue, thereby allowing optimized hormonal NIS stimulation for potential diagnostic and therapeutic applications.

The iodide-concentrating activity of the thyroid gland allows the use of radioiodine for diagnosis of thyroid nodules by thyroid scintigraphy as well as ablation of postsurgical remnants and treatment of recurrent and metastatic disease in thyroid cancer. The detection and characterization of mgNIS suggests that radioiodine might serve a similar function in the diagnosis and treatment of breast cancer (Fig. 3). Long before the cloning of NIS and identification of mgNIS it was known that breast atypia and malignancy reveal increased radioiodine uptake, and breast cancers can be detected by radioiodine/¹³¹I Tc scintigraphy (43–45). One study even indicated that high radioiodine uptake may prove to be the most specific biochemical characteristic of hormone-dependent breast tumors compared with hormone-independent tumors (46). Although normal mammary gland epithelial cells express mgNIS physiologically only during late gestation and lactation, Tazebay et al. (32) demonstrated functional expression of mgNIS in experimental mammary adenocarcinomas in nongestational and nonlactating female transgenic mouse models carrying either an activated Ras oncogene (c-Ha-ras) or overexpressing the neu oncogene (c-erbB-2) by scintigraphic imaging and immunoblot analysis. Furthermore, using 3 polyclonal and monoclonal antibodies directed against different portions of the hNIS protein, mgNIS protein expression was examined in human breast tissue specimens, including 8 normal specimens from reductive mammoplasties, 29 malignant specimens (23 invasive carcinomas and 6 ductal carcinomas in situ), 13 extratumoral specimens, and 3 biopsies from pregnant women with breast nodules. Twenty of the 23 (87%) invasive carcinomas, and 5 of the 6 (83%) ductal carcinomas in situ were found to express mgNIS compared with only 3 of the 13 (23%) extratumoral specimens from tissue in the vicinity of the tumors. In addition, none of the 8 normal samples expressed mgNIS, whereas all 3 gestational samples were mgNIS positive. In contrast to the distinct mgNIS-specific immunoreactivity at the basolateral membrane of epithelial cells in lactating mammary gland, in malignant breast cells mgNIS-positive staining was localized both at the plasma membrane and intracellularly (32). The high prevalence of mgNIS in human breast cancer (>80%) indicates that mgNIS is up-regulated with high frequency during malignant transformation in human breast tissue and, therefore, has potential diagnostic value (Fig. 3). The demonstration of functional activity of mgNIS protein expressed in breast cancer tissue in 2 different transgenic mouse models further suggests that radioiodine may be a possible alternative diagnostic and therapeutic modality in breast cancer (Fig. 3). However, further studies in a larger series of patients are needed to confirm these
findings and to evaluate radioiodine as a new option in the diagnosis and treatment of breast cancer.

In addition to several studies demonstrating stimulation of functional mgNIS expression by PRL and oxytocin, a very recent report by Kogai et al. shows induction of NIS gene expression and radioiodine uptake in breast cancer cells after treatment with RA (47). RA plays a well characterized role in development, differentiation, and cell growth and may have a role in the treatment of a number of cancer types by inhibiting cell cycle progression and induction of apoptosis. In addition, RA has been shown to up-regulate NIS mRNA expression in human follicular thyroid carcinoma cell lines (24). In the estrogen receptor-positive human breast cancer cell line MCF-7 all-trans-retinoic (tRA) acid treatment stimulated iodide uptake in a time- and dose-dependent fashion up to approximately 9.4-fold. Stimulation with selective retinoid compounds indicated that induction of iodide uptake is mediated by the RA receptor, which is expressed in MCF-7 cells. In addition, treatment with tRA significantly stimulated NIS mRNA as well as NIS protein expression. In contrast, no induction of iodide uptake was observed after RA treatment of the estrogen receptor-negative human breast cancer cell line MDA-MB 231 or the normal human breast cell line MCF-12A. The absence of stimulation of iodide uptake after RA treatment of several other human cancer cell lines, including prostate cancer cells (LNCaP), choriocarcinoma cells (JEG-3), and lung cancer cells (A549 and H460), showed that the iodide uptake-stimulating effect of RA was cell selective. Although no iodide organification could be detected in tRA-treated MCF-7 cells, iodide efflux was slow compared with iodide efflux in FRTL-5 thyroid cells, which may be the result of the absence of pendrin in MCF-7 cells. Most importantly, an in vitro clonogenic assay demonstrated selective cytotoxicity of 131I in MCF-7 cells after tRA treatment (47). If these results can be confirmed in vivo, stimulation of radioiodine uptake by systemic retinoid treatment may have a role in the imaging as well as the therapy of breast cancer (Fig. 3).

**NIS as a novel therapeutic gene**

Thyroidal expression of NIS is responsible for the effective treatment of differentiated thyroid cancer, even in advanced metastatic disease, by radioactive iodine administration. The recent cloning and characterization of the hNIS gene (2, 3) have paved the way for the development of a novel cytoreductive gene therapy strategy for the treatment of thyroidal and extrathyroidal malignancies. 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. Early studies in transformed rat thyroid cells (FRTL-Tc) without iodide transport activity showed that transfection with rat NIS cDNA using electroporation is able to restore radioiodine accumulation in vitro and in vivo. FRTL-Tc cells stably expressing the rat NIS gene accumulated 125I 60-fold in vitro, and xenotransplants in Fischer 344 rats derived from the same stably transfected cell line trapped up to 27.3% of the total 125I dose with an effective half-life of approximately 6 h. The application of a therapeutic dose of 1 mCi 131I, however, did not result in a statistically significant tumor volume reduction (48). Expression of functionally active NIS has also been reported in human glioma cells in vitro and in vivo using adenovirus-mediated NIS gene delivery. Glioma cells showed an up to 112-fold increase in radioiodide uptake activity after infection with an adenovirus carrying the human NIS gene linked to the cytomegalovirus promoter. In a human glioma xenograft mouse model, intratumoral injection of this adenovirus resulted in functional NIS protein expression in vivo with an up to 25-fold increase in radioiodide accumulation compared with that in spleen (49). Furthermore, Mandell et al. demonstrated in vitro and in vivo iodide accumulation in several cancer cell lines, including melanoma, liver, colon, and ovarian carcinoma cells, after retrovirus-mediated transfection with the rat NIS gene. An in vitro clonogenic assay was used to demonstrate that rat NIS-transduced cancer cell lines can be selectively killed by the accumulated 131I. Rat NIS-transduced melanoma xenografts established in athymic nude mice accumulated significantly more 123I (6.9-fold increase) than nontransduced tumors (50). Similar results were obtained by Boland et al., who expressed the rat NIS gene in several tumor cell lines (cervix, prostate, breast, lung, and colon carcinoma cells) by adenovirus-mediated gene transfer in vitro and demonstrated radioiodide uptake (125- to 225-fold increase) as well as a selective cytotoxic effect of trapped radioiodide in vitro. In cervix and breast cancer xenografts, radioiodide uptake could also be demonstrated after in vivo NIS gene delivery. On the average, 11% of the total 125I dose could be recovered per g adenovirus-infected tumors. Intraperitoneal application of therapeutic doses of only up to 90 μCi 131I did not result in a therapeutic response (51). In a very recent study, Nakamoto et al. stably transfected a human breast cancer cell line with the rat NIS gene using electroporation and demonstrated a 44-fold increase in radioiodide uptake in vitro. Xenografts in athymic nude mice accumulated 16.7% of the total 125I dose (52). These data demonstrate the potential of NIS gene transfer to induce iodide accumulation activity in tumor cells, although the therapeutic efficacy of accumulated radioiodine remains to be confirmed in vivo.

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 (53). Recently, prostate cancer (LNCaP) cells were shown to be selectively killed by accumulated 131I after induction of tissue-specific iodide uptake activity by prostate-specific antigen promoter-directed NIS expression in vitro. Iodide accumulation has been confirmed in vivo in LNCaP cell xenografts in athymic nude mice and has been high enough to allow a therapeutic effect of 131I in vivo. A single therapeutic 131I dose of 3 mCi was administered and was shown to elicit a dramatic therapeutic response in NIS-transfected LNCaP cell xenografts, with an average volume reduction of more than 90% and complete tumor regression in up to 60% of the tumors (54, 55). These studies clearly show for the first time that NIS gene delivery into nonthyroidal tumors is capable of inducing the accumulation of therapeutically effective radioiodine doses and might therefore represent an effective and potentially curative therapy for extrathyroidal tumors, in particular...
prostate cancer (54, 55). In addition to its therapeutic application, tissue-specific NIS gene transfer in extrathyroidal tumors could provide an important diagnostic tool for imaging nonthyroidal tumors and their metastases using $^{99m}$Tc or $^{123}$I (Fig. 4).

Cloning and characterization of the NIS gene, therefore,
clearly offer the possibility of a novel gene therapy strategy based on NIS gene transfer into nonthyroidal tumor cells, followed by radioiodine therapy. Before clinical application of the NIS gene as a novel therapeutic gene, several hurdles remain to be surmounted, and a number of open questions need to be addressed. The therapeutic efficacy of $^{131}$I depends on the radiosensitivity of the tumor tissue and the biological half-life of $^{131}$I, which is dependent on the extent of radioiodine trapping and its retention time in the tumor. One major argument that is frequently raised against the feasibility of using radioiodine therapy after NIS gene transfer into nonthyroidal tumors is the general assumption that the success of radioiodine treatment of thyroid cancer is dependent on NIS-mediated trapping as well as organification of the trapped radioiodine. Although there are reports of iodide organification in nonthyroidal tissues (27, 28), this process may not be carried out as efficiently as in thyroid tissue, thereby potentially limiting the retention time and radiation dose delivery of radioiodine. However, no clear data exist to support the hypothesis that organification is a prerequisite of radioiodine treatment efficacy. In fact, it has been shown that even thyroid carcinomas and their metastases often reveal a reduced capacity for iodide organification and thyroid hormone synthesis due to disrupted follicular architecture and function and lack of Tg expression (56, 57). Organification of trapped radioiodine is certainly capable of increasing the achieved radiation dose by enhancing the retention time and biological half-life of radioiodine in the target tissue and, if necessary, might be used therapeutically in nonorganifying tissues by coupling NIS gene transfer with delivery of the TPO or Tg gene. Because accumulated radioiodine is not organified in LNCaP cells, the above-summarized data for LNCaP cell xenografts (55) indicate clearly that iodide organification is not required to achieve a therapeutic effect of radioiodine in tumor tissue.

Thyroid follicular cells also possess an apical transporter for iodide into the thyroidal colloid, which removes iodide from the cytoplasm. Recently, this function has been shown to be mediated, at least in part, by pendrin, which is a chloride/iodide transporter (7–9, 58) (Fig. 2). In contrast to thyroid follicular cells, there may be no efficient transport mechanism for iodide efflux out of nonthyroidal cells that do not normally transport and metabolize iodide. Thus, the radioiodine retention time in NIS-transfected nonthyroidal tumors may be enhanced by the lack of an effective efflux mechanism. Further studies are needed to address these efflux mechanisms in NIS-transfected nonthyroidal tumor cells, as they represent important factors contributing to the biological half-life of trapped radioiodine, and its pharmacological modulation might offer the prospect of increasing radioiodine retention time in nonthyroidal cells. Lithium, for example, has been shown to reduce iodide efflux from thyroidal cells, thereby increasing radioiodine retention and enhancing the therapeutic efficacy of radioiodine in the treatment of Graves’ hyperthyroidism (59). Further studies are needed to address the exact molecular mechanisms of lithium-mediated reduction of iodide efflux in the thyroid gland as well as a possible effect of lithium on nonthyroidal iodide efflux.

The next crucial step toward clinical application of NIS gene delivery followed by radioiodine therapy in nonthyroidal cancer patients will involve the generation and investigation of in vivo gene delivery systems such as adenoviral and retroviral vectors. A central theme of such studies will be attempts to achieve safe and efficient gene delivery systems using vectors that can be administered systemically and in which gene expression is regulated in a tissue-specific fashion.

**Summary**

As the thyroidal membrane protein that mediates iodide transport into thyroid follicular cells, NIS plays a key role in thyroid pathophysiology and allows very effective use of radioiodine for diagnosis and therapy of thyroid cancer. Since NIS was cloned in 1996, most studies have demonstrated decreased NIS expression levels in thyroid carcinomas, which may account at least in part for the reduced iodide uptake activity generally observed in such tumors. Initial therapeutic strategies, including RA and demethylation treatment, have been explored with the aim of stimulating NIS expression and optimizing therapeutic responsiveness to $^{131}$I in thyroid cancer.

Functional NIS expression has further been detected and characterized in lactating mammary gland, providing iodide to the newborn, as well as in breast cancer tissue in vitro and in vivo, suggesting that radioiodine may be a potential alternative diagnostic and therapeutic modality in breast cancer. Although a recent study using RA to stimulate functional NIS expression in breast cancer cells yielded a selective cytotoxic effect of $^{131}$I in vitro, in vivo studies are needed to confirm these findings.

In addition, cloning and molecular analysis of the NIS gene offer the possibility of a novel cytoreductive gene therapy strategy based on targeted NIS gene transfer into nonthyroidal tumors, followed by radioiodine therapy. This novel form of gene therapy would extend the application of carrier-free radioiodine and the extensive experience with radioiodine in thyroid cancer management to the treatment of extrathyroidal tumors. If further in vivo studies using efficient and safe in vivo NIS gene delivery systems can confirm the promising preliminary results obtained in various in vitro and in vivo experiments, this approach is undoubtedly one of the most exciting chapters of NIS gene-based research since its cloning in 1996.

**References**

POTENTIAL ROLE OF SODIUM IODIDE SYMPORTER in CANCER THERAPY

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