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INVITED REVIEW

A perspective view of sodium iodide symporter research and its clinical implications

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Abstract

The sodium iodide symporter (NIS) is an intrinsic plasma membrane protein that mediates active iodide transport into the thyroid gland and into several extrathyroidal tissues, in particular the lactating mammary gland. Cloning and molecular characterization of the NIS have allowed the investigation of its key role in thyroid physiology as well as its potential pathophysiological and therapeutic implications in benign and malignant thyroid diseases. Similarly, elucidating the mechanisms underlying the regulation of NIS in lactating mammary gland and breast cancer, in which more than 80% of cases express endogenous NIS, may lead to findings that have novel implications for pathophysiology and therapy. Two approaches may, in the future, pave the way to extend the use of radioiodide treatment to nonthyroidal cancer. One is based on the reinduction of endogenous NIS expression in thyroid and breast cancer by targeting the main mechanisms involving tumoral transformation and dedifferentiation. The other is based on the application of NIS as a novel cytoreductive gene therapy strategy. NIS offers the unique advantage that it can be used both as a reporter and as a therapeutic gene, so that it is possible to image, monitor, and treat the tumor with radioiodide, just as in differentiated thyroid cancer. This review summarizes the main recent findings in NIS research that have a direct impact on diagnosis and therapeutic management.

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Introduction

The ability of the thyroid gland to concentrate iodide has long provided the basis for diagnosis and therapeutic management of benign thyroid diseases and thyroid cancer. As early as 1896, Baumann found for the first time that the thyroid gland concentrates iodide by a factor of 20–40 times with respect to plasma under physiological conditions (1). Given the extraordinary importance of iodide in the thyroid, a large body of research followed this discovery, increasing our knowledge of thyroid gland physiology and pathology. Another important breakthrough occurred in 1942, when, in the Massachusetts Institute of Technology (MIT), taking advantage of this unique iodide-concentrating characteristic, radioactive iodide was supplied to patients in order to image and, eventually, destroy their thyroid glands (2, 3). Since then, thousands of patients have been given radioiodide for medical purposes, improving significantly the management of thyroid diseases. A third breakthrough occurred in 1996 when the sodium iodide symporter (NIS) was finally cloned, first in rats (4) and then in humans (5). This has improved our understanding of thyroid pathophysiology tremendously, and has opened a promising field in cancer research; it has opened the way for new strategies to reexpress endogenous NIS in thyroid and breast cancer, as well as to induce ectopic functional NIS expression by gene transfer in nonthyroidal cancers (6). Radioiodide treatment that formerly was beneficial only in thyroid diseases can eventually be extended to a wide range of nonthyroidal cancers.

NIS is an integral plasma membrane glycoprotein that mediates active transport of iodide into the follicular thyroid cell, the first step in thyroid hormone synthesis. The symporter cotransports two sodium ions(Na^+) along with one iodide ion(I^-), with the transmembrane sodium gradient serving as the driving force for iodide uptake. The electrochemical sodium gradient that allows NIS to be functional is maintained by the oubaine-sensitive Na^+/K^+-ATPase. NIS-mediated iodide transport is inhibited by the competitive inhibitors thiocyanate and perchlorate. Inside the cell, iodide is driven through the apical membrane to the follicular lumen by pendrin (the Pendred syndrome gene product) (7) and other unknown systems, in a process called iodide efflux. Thus, cell polarization plays a central role in correct iodide transport. The formerly named apical iodide transporter (AIT), currently known as Na^+/monocarboxylate transporter (SMCT), has been

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proven not to transport iodide in thyroid cells (8). In a very complex process, thyroperoxidase (TPO) catalyzes an oxidative reaction consisting of iodide oxidation, its binding to tyrosine residues of thyroglobulin (Tg), and the oxidative coupling of two iodotyrosines will further provide triiodothyronine (T_3) and thyroxine (T_4). Thyroglobulin is a large protein that accumulates in the thyroid colloid and this process is called iodide organification and can be inhibited by propylthiouracil and methimazole, which are TPO enzyme-activity inhibitors. TPO has no biological activity in the absence of H_2O_2, which is likely to be the limiting factor for Tg iodination when iodide supply is normal. The H_2O_2-generating system is a calcium dependent and transmembrane system, whose main enzymes are thyroid oxidase (ThOX)1 and ThOX2 also called 'Duox' for dual oxidase) belonging to the nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase (NOX) family and have been recently cloned (9). After endocytosis and phagolysosomal degradation of thyroglobulin, T_3 and T_4 are released to the plasma. The entire process, including iodide transport and NIS expression, is regulated by thyroid-stimulating hormone (TSH), which interacts with the TSH receptor (TSHR; a seven-transmembrane receptor coupled to heterotrimeric G-protein) localized in the basolateral membrane, and stimulates cAMP-related pathways.

The human *NIS* gene is localized on chromosome 19p12–13.2, and encodes a glycoprotein of 643 aminoacids (aa) with a molecular mass of approximately 70–90 kDa. The gene comprises 15 exons interrupted by 14 introns and has an open reading frame of 1929 nucleotides. As a member of the solute carrier family (SLC5A5, according to the Online Mendelian Inheritance in Man (OMIM) classification; MIM number: 601843), all of which use the electrochemical sodium gradient as the driving force, NIS is predicted to have a 13-transmembrane segment pattern with the N-terminus facing the extracellular milieu, and the COOH-terminus facing the cytosol (10). There are three sites of glycosylation in the mature form of NIS, but glycosylation does not seem to play any role in the stability, activity, or targeting to the membrane of the symporter (11). NIS is also a phosphoprotein, and the major phosphorylation region is the COOH terminus (12). The purpose of this review is to summarize the most recent findings on NIS research emphasizing its diagnostic and therapeutic implications.

Regulation of NIS

Main factors that regulate NIS expression

TSH TSH is the main hormonal regulator of thyroid function, and the majority of its actions are mediated by activation of adenylate cyclase through the GTP-binding protein, Gαs. It has long been established that TSH stimulates iodide transport into the thyroid cells via the cAMP pathway in a protein synthesis-dependent manner (13, 14). After the cloning of NIS, further studies revealed that at least two mechanisms are used by TSH through cAMP to regulate iodide transport. The first and most obvious one positively regulates *NIS* gene transcription, as demonstrated in studies *in vitro* (15, 16) and *in vivo*. The second one induces the activity of the NIS protein (12, 17), presumably by post-translational modifications, which are essential for NIS trafficking to the membrane, and will be commented on in detail later.

Iodide Apart from TSH, the main factor regulating the accumulation of iodide in the thyroid and, thus, NIS activity, has long been considered to be iodide itself. It is a well-known phenomenon among physicians that high doses of iodide cause an acute and transient decrease in thyroid function and it is used occasionally to obtain rapid remission in severe hyperthyroidism. Wolff and Chaikoff reported in 1948 that organic binding of iodide (which later was shown to be mediated by TPO) in the rat thyroid *in vivo* was blocked when iodide plasma levels reached a critical high threshold, a phenomenon known as the acute Wolff–Chaikoff effect (18). Wolff *et al.* reported in 1949 that as early as 2 days after onset of this acute inhibitory effect, an escape or adaptation from the effect occurred, so that the level of organification of iodide was restored and normal hormone biosynthesis reappeared. This phenomenon constitutes a highly specialized intrinsic autoregulatory system that protects the thyroid from high doses of iodide, but at the same time ensures adequate iodide uptake for hormone biosynthesis (19).

The mechanism underlying the inhibition of iodide organification by high levels of iodide, the acute Wolff–Chaikoff effect remains poorly understood after all these years. Grollman *et al.* observed that the presence of methimazole (MMI), an inhibitor of TPO activity, abolished the iodide uptake-suppressing effect of iodide (20). This action suggested to the authors that the Wolff–Chaikoff effect is mediated by an intracellular iodinated compound, and they proposed the iodolipids as candidates, since these compounds have been shown to inhibit the TSH-mediated adenylate cyclase activity.

The cloning of *NIS* has provided new tools to improve our understanding of the escape from the Wolff–Chaikoff effect. One *in vivo* study reported that acute administration of iodide to rats decreased the NIS mRNA levels at 6 h and the protein levels at 24 h, and this inhibitory effect was maintained during chronic iodide ingestion (6 days). These results clearly suggest that escape is due to a decrease in NIS expression (21). The same group studied the effect of high doses of iodide on NIS regulation in FRTL-5 cells. Surprisingly, high

doses of iodide during 24 and 48 h did not decrease the NIS mRNA levels, but did decrease the NIS protein levels in a dose-dependent manner, suggesting that in this *in vitro* thyroid cell model, iodide modulates NIS at a post-transcriptional level (22). These results are in sharp contrast with the previous *in vivo* study reported by the same authors. Furthermore, in another study, a 50% decrease in both iodide uptake and NIS mRNA levels after iodide administration during 48 h was observed in FRTL-5 cells (23). Whether the different results concerning NIS regulation by iodide at a transcriptional and/or post-transcriptional level are due to different experimental conditions is something that needs to be clarified.

Cytokines and growth factors Cytokines, such as tumor necrosis factor (TNF)-α, TNF-β, interferon (IFN)-γ, interleukin (IL)-1α, IL-1β, and IL-6 have been proven to inhibit NIS mRNA expression and iodide uptake activity in FRTL-5 and human thyroid cells (24–26). One *in vivo* study observed significant inhibition of thyroidal NIS mRNA and protein expression in transgenic mice that develop hypothyroidism following thyroid-targeted expression of IFN-γ (27). The fact that all these cytokines exert inhibitory effects on NIS expression matches well with the mechanisms underlying autoimmune hypothyroidism, as T-cell recognition of autoantigens in the thyroid leads to a mixed pattern of cytokine production. Moreover, the thyroid cells also produce a number of proinflammatory molecules, which will tend to exacerbate the autoimmune process leading to hypothyroidism.

Transforming growth factor (TGF)-β, a potent inhibitor of growth and DNA synthesis in thyroid cells, decreased TSH-induced NIS expression as well as TSH-induced iodide uptake activity in FRTL-5 cells (25, 26). In contrast with other cytokines, TGF-β also induced a change in FRTL-5 cells from a cuboidal to a flattened morphology (25). Similarly, IGF-I also decreased TSH-induced NIS mRNA levels, although it has the opposite effect on TPO and thyroglobulin (Tg) mRNA levels (28).

Thyroglobulin Tg acts as a potent suppressor of thyroid-specific genes, including NIS mRNA levels and subsequent iodide uptake activity. The mechanism underlying this inhibitory effect is not well understood. One group has observed that suppression of thyroid-specific gene expression by purified 12S, 19S, and 27S follicular Tg was dependent on their ability to bind to the FRTL-5 membrane. This binding was blocked by an antibody against the thyroid apical membrane asialoglycoprotein receptor, which is a phosphoprotein that is critical for ATP-mediated inactivation of receptor-mediated endocytosis (29). Whether it acts directly or indirectly, Tg-mediated suppression of NIS expression may represent a negative feedback autoregulatory mechanism that counterbalances TSH stimulation of follicular function (30). This is in accordance with the particular distribution of NIS observed when immunohistochemistry (IHC) is performed in normal thyroid; there is a low or null NIS expression in bigger thyroid follicles (containing larger amounts of Tg), which show inactive and flattened epithelial cells, in contrast with the smaller follicles were NIS expression is increased and cells are cuboidal reflecting a higher activity in order to produce more Tg.

Estradiol Estradiol increases proliferation and downregulates NIS expression and iodide uptake in FRTL-5 cells, suggesting that this may contribute to the higher prevalence of goiter in women (31).

Signal transduction pathways

TSH–TSH-R-cAMP–protein kinase (PKA) signaling is the central pathway for thyroid proliferation and differentiation, in sharp contrast with other tissues where cAMP triggers inhibitory effects on proliferation. Thus, the integrity of this pathway is essential for NIS expression, although the exact mechanism by which PKA promotes NIS expression is still unknown. Additionally, PKA-independent signaling has been shown to play a complementary role in thyroid differentiation. In FRTL-5 cells, chronic stimulation with TSH downregulates PKA (32) and acute stimulation with cAMP agonists under these conditions results in increased *NIS* transcriptional activity without PKA activation (33). The MEK–ERK pathway has been proposed as a PKA-independent pathway, as MEK inhibition partially inhibits cAMP-induced *NIS* gene transcription (43%) (33), and this pathway seems to be stimulated by TSH through Rap-1 without PKA activation (34, 35). However, activation of the MAPK pathway in response to TSH/cAMP has not been confirmed by other groups, including ours (36, 37), leaving this issue as a matter of controversy. Another pathway, the p38 MAPK pathway, has been proven to have a stimulatory effect on *NIS* gene expression, although it seems to do it in a PKA-dependent way (38).

Recently, our group has shown that at least two inhibitory pathways are likely to counterbalance the aforementioned pathways, resulting in an overall balance (Fig. 1). The PI3K pathway downregulates NIS expression in thyroid cells. In FRTL-5 cells, we have demonstrated that IGF-I inhibits the cAMP-induced NIS expression through the PI3K pathway (28). Similarly, expression of a Ras mutant that selectively stimulates PI3K markedly decreases the TSH-induced NIS expression in WRT rat thyroid cells (39). The second inhibitory pathway is the TGF-β/Smad signaling. As

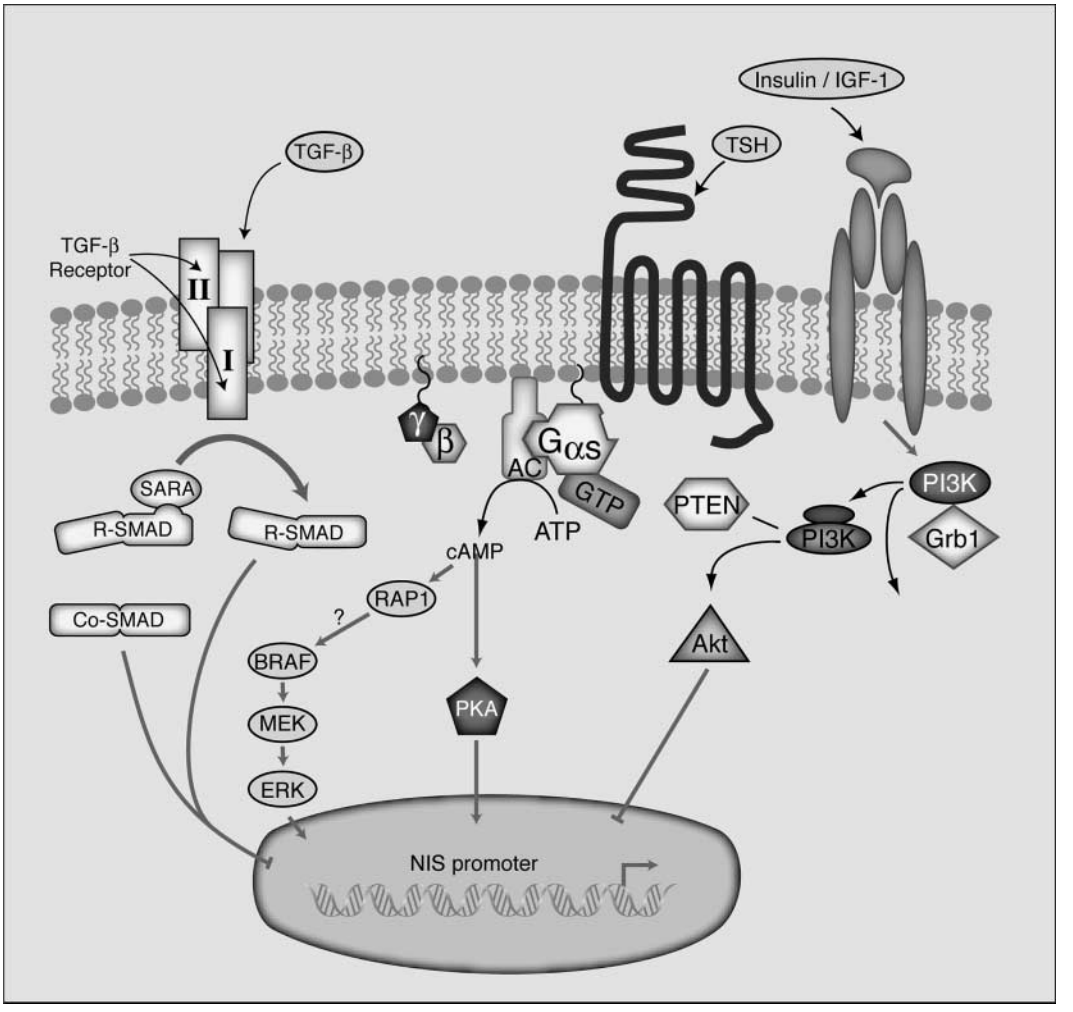

Figure 1 Signal transduction pathways involved in sodium iodide symporter (NIS) regulation. The figure shows the main stimulatory pathway based on thyroid-stimulating hormone receptor (TSH-R)–cAMP both protein kinase (PKA)-dependent and PKA-independent and the two inhibitory pathways described by our group based on insulin-like growth factor (IGF)-I–PI3K–Akt and transforming growth factor (TGF)-β-Smad, AC, adenylate cyclase.

mentioned earlier, TGF-β decreases TSH-induced NIS expression and iodide uptake activity in FRTL-5 cells (25, 26). Our group further demonstrated that TGF-β-induced downregulation of NIS is mediated via Smad by decreasing Pax-8 mRNA levels and impairing PAX-8 binding to the NIS promoter due to the physical interaction between Smad-3 and Pax-8 (40).

NIS transcriptional activity

Cell type-specific gene transcription is dependent on a set of transcription factors whose combination is unique to that cell type. Three transcription factors, thyroid transcription factor (TITF)-1, FOXE-1, and Pax-8, have been implicated in the control of the genes encoding thyroid-specific proteins, such as thyroglobulin (Tg), thyroid peroxidase (TPO), and TSH receptor (TSH-R). TITF-1 is a homeodomain (HD)-containing protein, present in the developing thyroid, lung, and diencephalon (41). FOXE-1, a forkhead protein, has been detected in the endoderm of the developing foregut, including the thyroid anlage, and in the anterior pituitary (42), while the paired-domain (PD) factor, PAX-8, is present in thyroid, kidney, and the central nervous system (43).

The structures of Tg and TPO promoters are very similar to each other. TITF-1 and PAX-8 bind to a specific region of both promoters, and DNA sequences recognized by both factors largely overlap (44). TITF-1 and PAX-8 could be used as alternatives to each other, depending on the functional requirements of Tg and TPO promoters (45). On the other hand, the structure of the TSH-R promoter is different from Tg and TPO (46). The TSH-R promoter lacks the PAX-8-binding site, and gene expression depends on TITF-1 and other factors that interact with a cAMP-response element (CRE) (47).

The structure of the rat NIS promoter has been studied in detail in the past few years. There are two main regulatory regions: the proximal NIS promoter localized between nucleotides −124 and −420 relative to the transcription start site, and the NIS upstream enhancer (NUE) localized between nucleotides −2260 and −2495 (16, 33) (Fig. 2). The NIS proximal promoter contains binding sites for TITF-1 (48), NTF-1 (NIS–TSH-responsive factor-1) (49), and Sp-1 (50), whereas the NUE contains binding sites for PAX-8 and TITF-1 and cAMP-responsive element (CRE)-like sequences. Although the role and importance of these regions and factors need further investigation, it is clear that the NUE is essential for NIS transcriptional activity and that the functional interaction between PAX-8 and a factor binding at the CRE-like sequence is necessary to achieve thyroid-specific transcription in a cAMP-dependent manner. By contrast, TITF-1 seems not to be necessary to induce NUE-dependent transcription (16, 33).

Post-transcriptional regulation of NIS

NIS needs to be localized in the plasma membrane to be fully functional. This is important not only for iodide transport into the thyroid gland, but also for radioiodide therapy in thyroid cancer. The decrease in iodide uptake observed in most thyroid cancers is due to impaired NIS targeting of, or retention at, the plasma membrane (see section NIS and thyroid cancer). Therefore, it is of considerable interest to elucidate the mechanisms that regulate the subcellular distribution of NIS).

After TSH deprivation, cultured rat thyroid cells still express NIS for several days due to its long half-life (3–5 days), but it is no longer retained in the membrane and iodide transport decreases dramatically within the first 24 h. This suggests that there are TSH-dependent mechanisms other than the transcriptional ones that keep NIS at the plasma membrane in thyroid cells (12). As NIS is a phosphoprotein and phosphorylation has been reported to play a role in regulating the targeting of other transporters, this mechanism has been postulated to be responsible for TSH-mediated post-transcriptional regulation of NIS. In fact, its phosphorylation pattern seems to be partially modulated by TSH in a PKA-dependent manner. (12). However, whether NIS phosphorylation is involved in trafficking and/or retention in the basolateral plasma membrane is something that has not yet been demonstrated. NIS also contains several sequences involved in protein–protein interactions. For instance, NIS contains a PDZ-target motif at the COOH-terminal tail that is recognized by PDZ-binding proteins, which have been implicated in regulating the internalization of other transporters. Other target motifs are also present at the COOH-terminal tail, such as a dileucine motif, which interacts directly with the clathrin-coated machinery (6).

NIS and benign thyroid disease

Congenital hypothyroidism (CH), a disease with an incidence of 1 in 3000 in neonates, has an irreversible deleterious effect on the development of the newborn leading to neurological defects if not treated. The most common cause of permanent CH is thyroid dysgenesis (85–90%) in which the transcription factor genes, *TITF-1*, *FOXE-1*, and *PAX-8*, play an obvious role in the etiology. Inborn errors of thyroid hormonogenesis are responsible for 10–15% of CH cases and usually have autosomal recessive inheritance, consistent with a single gene mutation. Mutations in thyroid-specific

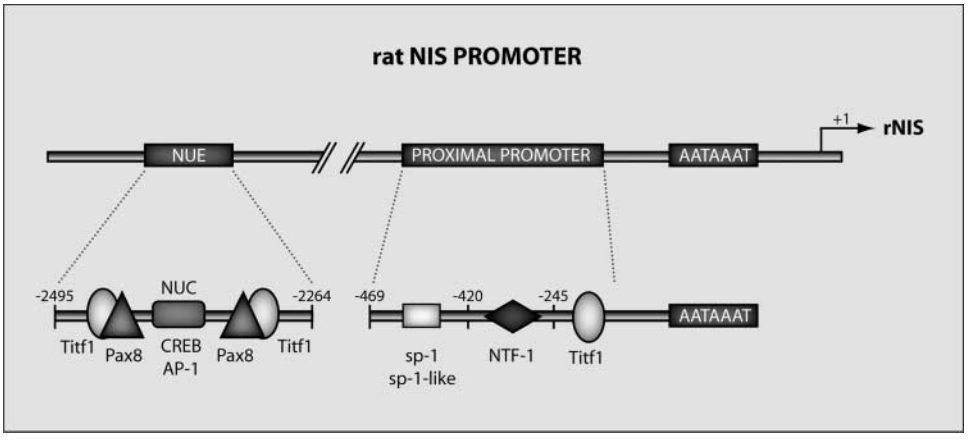

Figure 2 Diagram of the rat sodium iodide symporter (NIS) promoter showing the major transcription start site +1 and the proximal promoter and the NIS upstream enhancer (NUE). The proximal promoter contains binding site for thyroid transcription factor-1(TITF-1), NIS–thyroid-stimulating hormone-responsive factor (NTF)-1, and Sp-1. The NUE contains two Pax-8 and two TITF-1-binding sites as well as a cAMP-response element (CRE)-like binding site.

genes, such as *TPO*, *Tg*, and *TSH-R* and more recently, *THOX* and *Pendrin*, have been identified among causes of congenital hypothyroidism. Iodide transport defect (ITD; OMIM 274400) is a cause of CH mediated by NIS mutations with an autosomal recessive inheritance pattern. After the cloning of *NIS*, 11 mutations in this symporter have been identified so far: G93R, Q267E, C272X, T354P, 515X, Y531X, G543E, G395R, V59E, deletion 143–323, and deletion 439–443 (51–59). They are either nonsense, alternative splicing, frameshift, deletion, or missense mutations of the *NIS* gene. A variable degree of hypothyroidism, goiter, reduced or absent iodide uptake and low iodide saliva:plasma ratio was observed in all these patients.

Although the clinical picture of these patients has been well described, only four mutations have been characterized at the molecular level to date: T354P, G395R, Q267E, and recently, G543E. The mechanisms underlying the lack of activity of these four mutants have provided some new insights, but still there is a long way to go to decipher the structural and functional mechanisms by which NIS is able to cotransport two Na^+ and one I^-. The hydroxyl group at residue 354 was shown to be essential for NIS function (57), and the presence of a small and uncharged aa residue at position 395 is required for NIS activity (60). Although one study based on flow cytometry using a monoclonal NIS antibody found impaired trafficking to the membrane of the Q267E mutation (61), a more extensive study using the same monoclonal NIS antibody and several approaches (biotinylation, flow cytometry, and immunofluorescence) conclusively demonstrated that Q267E NIS was properly targeted to the plasma membrane. In fact, the authors found this mutant decreased (but did not abolish) iodide uptake by lowering the turnover number of NIS, or in other words, by decreasing the maximal rate of transport activity (58). None of these three aforementioned mutations affected NIS expression or trafficking to the plasma membrane. Interestingly, the G543E mutation, located on the cytoplasmic side of transmembrane segment XIII, impairs NIS maturation and trafficking to the membrane, and NIS is retained intracellularly, presumably because of aberrant folding (59).

Many studies have focused on the potential role of NIS as a novel autoantigen in the pathogenesis of autoimmune thyroid disease (AITD). At least two reviews have summarized all the results obtained from these studies (6, 62) in detail, and there are no recent advances in this area. Briefly, both reviews conclude that, although there is evidence that autoantibodies directed against NIS are present in sera from patients with AITD, the biological effect of anti-NIS autoantibodies remains unclear and NIS does not seem to play a major role as an autoantigen.

NIS and thyroid cancer

Iodide uptake is diminished in thyroid cancer compared with normal thyroid tissue. Induction of hypothyroidism to elevate endogenous TSH or administration of human recombinant TSH is used by physicians to enhance iodide uptake, proving that cancer cells still maintain a certain degree of differentiation and hormonal responsiveness. When thyroid cancer undergoes total loss of differentiation, no iodide uptake is observed, and the prognosis is clearly worse. Overall, low or absent NIS expression was thought to occur under these circumstances. Unexpectedly, after the cloning of *NIS*, studies based on IHC and reverse transcriptase (RT)-PCR revealed that up to 70–80% of thyroid cancers expressed or even overexpressed NIS, yet this expression was mainly cytoplasmatic and not targeted to the basolateral membrane (63–65). These observations pointed out that targeting of, and retention in, the plasma membrane are essential for NIS to be fully functional, explaining why iodide uptake is diminished in thyroid cancer (Fig. 3).

In an attempt to explore the use of NIS as a biological marker in thyroid cancer, a large retrospective study was performed in order to determine whether NIS protein expression in primary differentiated thyroid cancer tumors correlated with the subsequent ^{131}I uptake by metastatic lesions in the same patients. Although the authors found that positive NIS immunoreactivity in primary tumors seemed to be predictive of subsequent recurrences positive in ^{131}I scans, this study did not take into account whether NIS was expressed in the membrane and negative NIS staining did not predict ^{131}I scan-negative metastasis (66). Another study demonstrated that low NIS expression assessed by RT-PCR correlated with more aggressive tumors (67). In accordance with these data is our recent study, where BRAF-positive papillary thyroid carcinoma correlated with scan-negative recurrences and impairment of NIS targeting to the membrane as assessed by IHC (68). Overall, NIS expression may have a role as a new biological marker in the post-operative management of patients with differentiated thyroid carcinoma, but these findings need further confirmation in prospective studies.

Oncogenic mutations of *BRAF* and *RAS* family genes as well as *RET* rearrangements play an important role in malignant transformation and tumor progression of thyroid cancer. These oncoproteins contribute to the partial or complete loss of differentiation of thyroid cancers, where a favorable prognosis highly depends on the degree of differentiation. Studies *in vitro* have found that the activation of these three oncogenes decreases NIS mRNA levels among other thyroid-specific genes (69–71). Interestingly, we further demonstrated that BRAF not only decreases NIS protein expression, but also impairs NIS targeting to the membrane both *in vitro* and *in vivo* (68). This finding is concordant with the

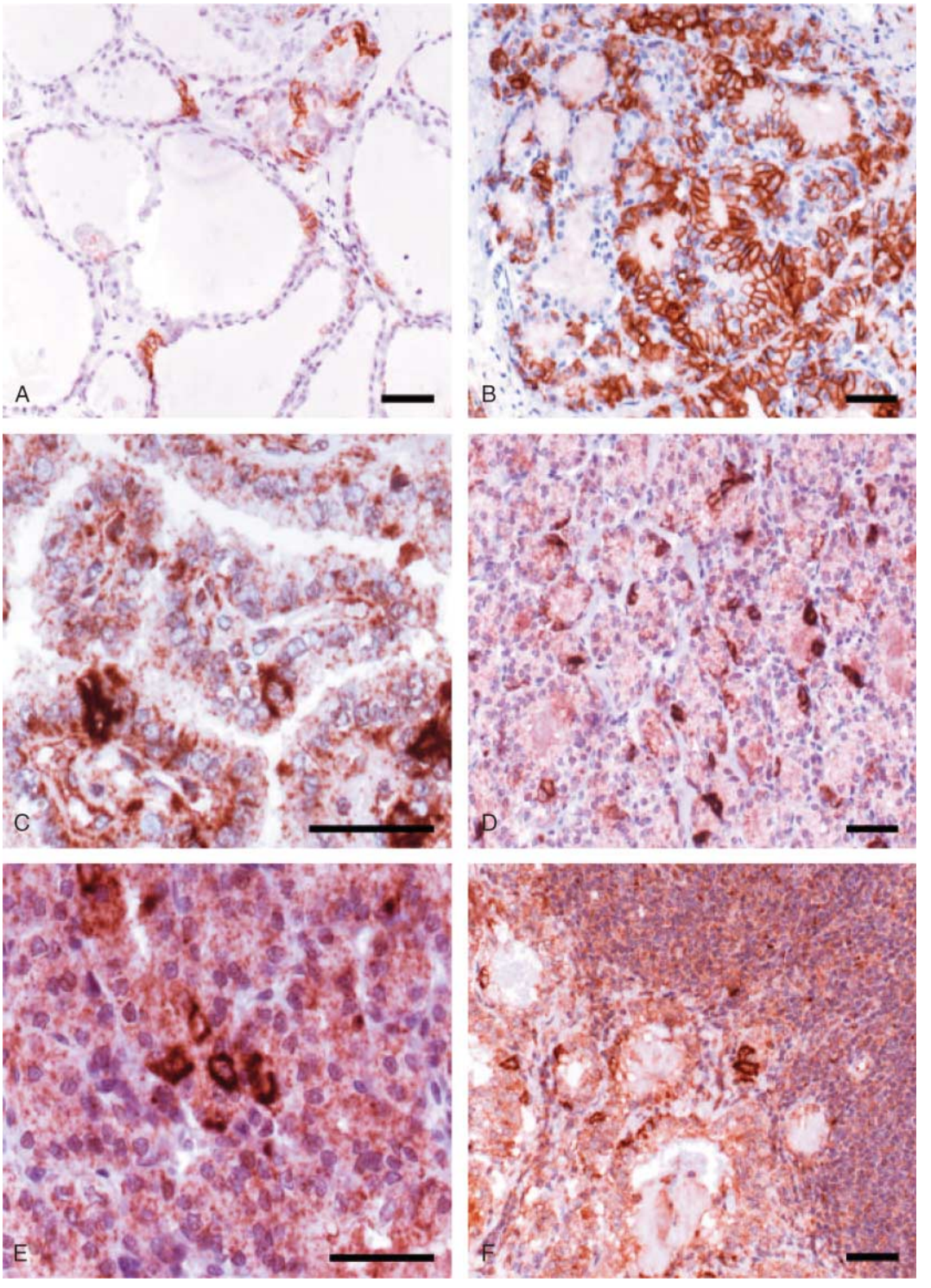

Figure 3 Sodium iodide symporter (NIS) expression in several samples of papillary thyroid cancer (PTC). Four micrometer sections were probed with anti-NIS Ab. (A) Normal thyroid tissue showing plasma membrane immunoreactivity in 10–20% of the follicular cells (original magnification 20×). (B) Graves disease showing follicular hyperplasia and predominant plasma membrane staining in more than 90% of the cells, used as positive control (original magnification 20×). (C) Classic PTC with intracellular immunoreactivity and distinct plasma membrane in >10% of the cells (original magnification 40×). (D) Follicular variant of PTC with intracellular immunoreactivity and distinct plasma membrane in >10% of the cells (original magnification 20×). (E) Several cells with distinct plasma membrane in a follicular variant of PTC (original magnification 40×). (F) Linfatic node with metastases of PTC showing distinct plasma membrane immunoreactivity (original magnification 20× (adapted from our own data; 68)).

association between BRAF mutation and a high frequency of recurrences that have lost the ability of concentrating iodide, as reported by our group and Xing's group (68, 72).

Induction of endogenous NIS expression in thyroid cancer

Since thyroid cancer conserves a certain degree of differentiation, one logical approach is to redifferentiate the cell and reinduce endogenous NIS expression. Several compounds, known to have tumor-inhibitory effects, have partially succeeded in reaching this goal:

Retinoic acid (RA) RA is a vitamin A derivative that plays a central role in differentiation and cell growth. There are two types of nuclear receptors that mediate RA actions, the retinoic acid receptors (RAR) and the retinoid X receptors (RXR). RAR has three isoforms, α, β, and γ, with a specific pattern of development and tissue distribution. All *trans* RA (*t*RA), a 'pan-retinoid' (nonselective) ligand to all RAR isomers, has been proved to induce NIS mRNA in two thyroid cancer cell lines, yet iodide uptake studies were not performed (73). It is worth noting that the same authors observed the opposite effect in the normal thyroid cell line FRTL-5 (73). Several clinical trials have been done using *t*RA in order to increase radioiodide uptake and improve clinical outcome of patients with recurrent thyroid cancer (see review by Dohan *et al.*) (6). In general terms, radioiodide uptake was improved in 20–42% of the cases, but tumor shrinkage was observed in very few cases after $^{131}I^-$ treatment. The largest study was carried out on 50 iodide scan-negative patients: 26% had a significant increase in radioiodide uptake, but only 16% had reduced tumor volume (74).

Troglitazone This antidiabetic compound is a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist that, among others effects, inhibits cell proliferation and induces apoptosis. Although the role of these nuclear receptors in carcinogenesis remains to be elucidated, the rearrangement PPAR-γ/PAX-8 occurs frequently in the follicular thyroid carcinoma and in the follicular variant of papillary thyroid carcinoma (75, 76). This rearrangement results in an inactivation of PPAR-γ function. Troglitazone has been reported not only to inhibit proliferation and induce apoptosis but also, interestingly, to increase NIS mRNA in several thyroid cancer cell lines (77, 78). More studies are needed to confirm these promising results *in vivo*.

Histone deacetylase (HDAC) inhibitors Epigenetic modifications, such as histone deacetylation play an important role in the regulation of gene expression and are likely to be involved in carcinogenesis. Histones are the core protein components of nucleosomes and their acetylation status regulates, in part, gene expression. Deacetylated histones are generally associated with silencing of gene expression, and aberrant acetylation is associated with several solid tumors and hematological malignancies. Inhibitors of HDACs, such as depsipeptide, trichostatin A (TSA), or valproic acid (VPA), have been shown to inhibit transformed cell growth *in vitro* and *in vivo* and to induce differentiation and/or apoptosis in many tumor cells, and are thus proving to be an exciting therapeutic approach to cancer (79).

Depsipeptide (FR901228) is an HDAC inhibitor that significantly increases NIS mRNA and iodide uptake in several thyroid cancer cell lines, including anaplastic cancer cell lines (80). Interestingly, depsipeptide induces concomitant expression of TPO, Tg, and TITF-1 leading to efficient iodide accumulation and retention *in vivo*. This suggests that the induction of iodide organification and a decreased iodide efflux are also restored by depsipeptide. Although depsipeptide exhibited cytotoxic effects *in vitro*, tumor volume reduction in *in vivo* models has not yet been reported. TSA is another HDAC inhibitor that increases mRNA NIS levels in several cancer cell lines, although iodide uptake induced by TSA has not yet been confirmed (81). Finally, VPA, a class I selective histone deacetylase inhibitor widely used as an anticonvulsant, promotes differentiation in some poorly differentiated thyroid cancer cell lines but not in others. One group observed that VPA-induced *NIS* gene expression, NIS membrane localization, and iodide accumulation in NPA cells (poorly differentiated). However, in ARO cells (anaplastic), only induction of NIS mRNA was observed, and this was not followed by any change in iodide uptake (82). Another group observed that NIS and Tg mRNA levels were upregulated by 93–370% in three thyroid follicular cell lines, but remained unchanged in a papillary cell line. However, no iodide uptake was performed in this study. In addition, VPA was highly effective at suppressing growth, with apoptosis induction and cell cycle arrest being the underlying mechanisms (83).

Overall, HDAC inhibitors are a promising therapy in thyroid cancer. Several HDAC inhibitors are in clinical trials with cancer patients. They are well tolerated, cause accumulation of acetylated histones in peripheral mononuclear cells and tumors and, more importantly, have clinical activity with objective tumor regression.

Demethylating agents DNA methylation, another epigenetic modification, is a covalent modification of cytosine residues that occurs at the dinucleotide sequence CpG in vertebrates. Nearly, half of all human genes have CpG islands associated with transcriptional start sites. Unmethylated CpG islands are seen in highly

transcribed genes, whereas heavily methylated CpG islands inhibit transcription (84). Although overall DNA methylation is often decreased in cancers, CpG islands in critical gene promoter regions can become hypermethylated, resulting in loss of gene expression. Abnormal patterns of DNA methylation are observed consistently in human tumors, including benign and malignant human thyroid tumors (85, 86). The human *NIS* gene has three CpG-rich regions. One region is located in the promoter, extending upstream for about 100 base pairs from the transcription start site. Two additional CpG-rich sequences are present downstream from this region, one of them extending to the first intron, corresponding to the human NIS (hNIS) untranslated region, and the other one extending to the coding region within the first exon (86). In seven human thyroid carcinoma cell lines lacking hNIS mRNA, treatment with 5-azacytidine (a demethylating agent) and sodium butyrate (HDAC inhibitor) was able to restore hNIS mRNA expression in four cell lines and iodide transport in two of them. Investigation of methylation patterns in these cell lines revealed that successful restoration of hNIS transcription was associated with demethylation of hNIS DNA in the untranslated region within the first exon. However, when 23 human thyroid tumor samples were studied, analysis of DNA methylation in the three CpG-rich regions of hNIS failed to define specific methylation patterns associated with NIS transcriptional failure. Furthermore, although loss of this NIS expression corresponded with loss of radioiodide uptake, some thyroid carcinomas with positive hNIS mRNA expression did not concentrate iodide, suggesting additional post-transcriptional mechanisms for loss of hNIS function (86). Likewise, Neumann *et al.* observed a lack of correlation between NIS mRNA and protein expression and NIS promoter methylation in benign cold thyroid nodules (87).

NIS and breast cancer

During late pregnancy and lactation, active transport of iodide takes place in the mammary gland in order to provide an adequate supply of iodide to the newborn. This delivery of iodide is essential for sufficient thyroid hormone production and subsequent proper development of the newborn's nervous system, skeletal muscle, and lungs. Tazebay *et al.* characterized the NIS protein in the mammary gland (mgNIS), and demonstrated that NIS is exclusively present in the mammary gland during gestation and lactation but not in nonlactating mammary gland, in contrast with the constitutive expression of NIS in the thyroid (88). Although hormonal regulation of mgNIS is not well understood, estrogens, oxytocin, and prolactin seem to play an important role.

Towards the end of gestation, high levels of mgNIS expression and a substantial increase in $^{125}I^-$ transport are achieved, indicating that induction of mgNIS expression precedes suckling. Both prolactin and oxytocin, which are released simultaneously in response to suckling, enhance the expression of mgNIS after delivery. In fact, the combination of estrogen, prolactin, and oxytocin (in the absence of progesterone) led to the highest levels of mgNIS expression in ovariectomized mice that received a high amount of exogenous estrogen. Interestingly, estrogens need to be above a certain threshold for oxytocin and prolactin to enhance mgNIS expression, otherwise prolactin antagonizes the stimulatory effect of oxytocin and no NIS expression is observed (89). Accordingly, Cho *et al.* observed that radioiodide uptake into lactating mammary gland was partially inhibited by treatment with selective oxytocin antagonists or bromocriptine, suggesting that radioiodide uptake in the mammary gland is, at least in part, modulated by oxytocin and prolactin (90). In cultured mammary explants, other lactogenic hormones, such as insulin and cortisol have been proven to stimulate functional NIS expression (88).

Tazebay *et al.* also demonstrated, using *in vivo* scintigraphic imaging, functional mgNIS activity that could be inhibited by perchlorate in experimental mammary adenocarcinomas in nongestational and nonlactating female transgenic mice carrying either an activated *Ras* oncogene or overexpressing the *Neu* oncogene. Most importantly, they also examined human breast tissue specimens and found that 87% of the 23 invasive carcinomas and 83% of the six ductal carcinomas *in situ* expressed mgNIS, compared with only 23% of the 13 noncancerous samples adjacent to, or in the vicinity of, the tumors. In addition, none of eight normal samples from reductive mammoplasties expressed mgNIS. They detected both plasma membrane and intracellular staining in some malignant breast cells, in contrast with the distinct basolateral plasma membrane staining of rat lactating mammary gland tissues (88). Using immunohistochemical methods, Wapnir *et al.* studied NIS protein expression in 371 human breast samples. They found NIS positivity in 76% of invasive breast carcinomas, 88% of ductal carcinomas *in situ*, and 80% of fibroadenomas, whereas the majority of normal breast samples (87%) excluding gestational/lactational changes were negative. Both intracellular and plasma membrane immunoreactivity were observed in all types of breast cancer, although it was not quantified (65).

The high prevalence of mgNIS expression in human breast cancer (more than 80%) reported in the aforementioned studies indicates that mgNIS is upregulated with high frequency during malignant transformation of breast tumors, and therefore, has a potential diagnostic and therapeutic value. Moon *et al.* found that ^{99m}Tc-pertechnetate scintigraphy revealed positive uptake in 4 out of 25 patients with breast tumors that

further correlated with the mgNIS expression in the breast tumor (91). Furthermore, in another study, 27 women were scanned with ^{99m}TcO4 or $^{123}I^-$ to assess NIS activity in their breast carcinoma metastases. Thyroid suppression was implemented with T_3. NIS expression was evaluated in index and/or metastatic tumor samples by IHC. Iodide uptake was noted in 25% of NIS-expressing tumors (two out of eight). The remaining cases did not show NIS expression or activity. The authors also estimated, by dosimetry, the feasibility of radioiodide ablation of metastases and proposed that T_3/methimazole downregulation of thyroidal NIS makes $^{131}I^-$ radioablation of breast carcinoma metastases possible with negligible thyroid uptake and radiation damage (92).

In conclusion, although more extensive studies in humans are necessary, functional expression of endogenous NIS in breast cancer and its metastases has been documented in both experimental mice and humans. These findings have a tremendous potential for the use of radioiodide in the diagnosis and treatment of breast cancer, as discussed later.

Induction of endogenous NIS in breast cancer

Not only the nonselective *t*RA, but also isoform-selective RAR agonists have been investigated in breast cancer in order to reinduce endogenous and functional NIS expression. *t*RA stimulates *NIS* gene expression and iodide uptake in the estrogen receptor (ER)-positive human breast cancer cell line MCF-7. Moreover, systemic *t*RA treatment of immunodeficient mice with MCF-7 cell xenografts markedly stimulated NIS expression and iodide uptake (93). However, lack of sufficient iodide retention and the potential toxicity of *t*RA due to the high doses required, led the same authors to investigate additional agents to enhance NIS expression. On the one hand, they observed that an isoform-selective retinoid, RAR-β/γ agonist combined with 9-*cis* RA, an RXR agonist, were more efficient and had less adverse effects than nonselective retinoids. On the other hand, dexamethasone significantly enhanced RA-induced NIS expression and cell-selective cytotoxicity of $^{131}I^-$ in MCF-7 cells. The authors concluded that the combination of RAR-β/γ selective agonist and dexamethasone synergistically increased iodide uptake and retention *in vitro*, reducing the effective dose of RA required and suggesting higher efficacy of $^{131}I^-$ treatment (94). Similar results concerning this synergistic interaction between dexamethasone and RA were also obtained by another group (95). Additionally, the cardiac homeobox transcription factor Nkx-2.5 seems to be involved in the RA-mediated induction of NIS in MCF-7 cells and can also induce NIS expression and iodide uptake by itself (96). Dohan *et al.* reported that not only hydrocortisone, but also isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor, markedly stimulated *t*RA-induced NIS protein expression and plasma membrane targeting in MCF-7 cells. Interestingly, the stimulatory effect of IBMX on NIS expression was not mediated by phosphodiesterase inhibition but by purinergic signaling. By contrast, neither iodide nor cAMP had a significant effect on NIS expression in MCF-7 (97). Overall, further insights are needed to understand the regulatory factors of NIS expression in mammary cells, which markedly differ from the thyroid. Therapeutic efficacy in breast cancer based on radioiodide will likely depend on manipulating NIS regulation.

NIS in other tissues

Despite the high specificity of thyroid uptake, radioiodine concentration has been demonstrated in a variety of nonthyroidal tissues, including salivary and lacrimal glands, stomach, choroid plexus, ciliary body of the eye, skin, placenta, lactating mammary gland, and thymus. Iodide transport in nonthyroidal tissues is known to be mediated by an active iodide translocating system that maintains an iodide gradient between secreted fluid and serum (96). Thyroidal and nonthyroidal iodide transport activities share several functional similarities, such as inhibition by thiocyanate and perchlorate as well as generation of iodide concentration gradients of similar magnitude. However, nonthyroidal tissues do not transport in a TSH-dependent manner and do not have the ability to organify accumulated iodide (with the possible exception of the lactating mammary gland where iodide is organified by binding to caseins and other milk proteins in correlation with peroxidase activity) (98).

The placenta transfers iodide and small amounts of thyroid hormones from mother to fetus. The molecular mechanisms of iodide transport from mother to fetus are not clear. Several groups demonstrated NIS expression in normal human placenta, preferentially in cytotrophoblast cells (99, 100). NIS is also expressed in the JAr choriocarcinoma cell line and the iodide transport in these cells appears to be regulated by human chorionic gonadotropin (hCG), which exerts its effects on iodide uptake through the stimulation of both NIS mRNA and protein expression (101). The BeWo choriocarcinoma cell line not only expresses NIS and accumulates iodide, but also this transporter is located in the apical (maternal) membrane of the cells, consistent with functional studies in bicameral chambers. Moreover, this cell line also expresses pendrin, and efflux of iodide occurs through a DIDS (a nonspecific inhibitor of anion exchangers including pendrin)-sensitive anion exchanger. Based on these results, the authors hypothesize that the trophoblast accumulates iodide through NIS and releases it to the fetal compartment through pendrin (102).

Accumulation of radioiodine by salivary and lacrimal glands and the gastrointestinal tract, including gastric mucosa and colon, is a common finding when performing whole body scintigraphy in patients with thyroid cancer after thyroidectomy (103) and may play a role in some of the dose-related side-effects associated with radioiodine therapy (104). In the salivary and lacrimal glands, NIS protein has been detected in the basolateral membrane of all ductal epithelial cells (105, 106). Similarly, NIS immunoreactivity has been detected in the basolateral membrane of mucin-secreting epithelial cells in the gastric mucosa and in the basolateral membrane of the epithelial cells lining mucosal crypts in the colon mucosa (106). This suggests that NIS is involved in active iodine transport from the serum to the secreted fluid of these exocrine glands and in the pathogenesis of salivary, lacrimal, and gastrointestinal dysfunction following radioiodine therapy. In addition, it has been postulated that inorganic iodide secreted by these exocrine glands to their corresponding mucosa followed by oxidation to hypoiodite may act as an antimicrobial agent, offering mucosal protection against environmental microorganisms (106). However, one important question remains to be elucidated: whether NIS is involved in the absorption of iodide by the gastrointestinal tract, as NIS has not been found in the apical membrane of the epithelial cells of the gastrointestinal tract.

As expected, NIS has been detected in clinically known iodide transporting tissues, namely thyroid, salivary gland, and stomach. Surprisingly, in a large survey using IHC methods, the following tissues were found to express NIS: bladder mucosa, endometrial glands, renal distal and collecting tubules, bronchial epithelium, intrahepatic bile canaliculi, gallbladder mucosa cells, prostate epithelium, and pancreatic exocrine cells. Immunoreactivity was particularly strong in the endometrium, bladder, kidney, and bile canaliculi. Additional tissues were found to have NIS expression, although it was not constant: adrenal, epididymis, and small bowel (65). In the same study, NIS was also found in carcinomas involving the following organs: bladder, cervix, oropharynx, colon, lung, pancreas, prostate, skin including melanoma, stomach, ovary, and endometrium. In the vast majority of these tumors, NIS was detected predominantly in the intracellular compartment and the expression was weak compared with thyroid and breast cancer where NIS immunoreactivity is intense and seemingly over-expressed. Although the authors did not find NIS immunoreactivity in normal lung alveolar tissue, cervix, esophagus, ovary, spleen, and skin, 46–68% of the corresponding malignant tissues demonstrated weak immunoreactivity (65). More studies are needed to confirm these interesting results and to elucidate the mechanisms underlying the expression of NIS during the malignant transformation of these carcinomas.

NIS gene transfer

One of the most promising areas in NIS research is the possibility of using *NIS* as a novel therapeutic gene in human cancer in order to extend the use of radioiodide therapy to nonthyroidal cancer. Indeed, NIS has been successfully transferred to several cancer cell lines enabling these cells to concentrate iodide. Since Shimura and coworkers transfected successfully rat *NIS* cDNA into malignant transformed rat thyroid cells (FRTL-Tc) that ordinarily do not show iodide transport activity (107), a large body of evidence has shown the feasibility of inducing functional NIS expression in extrathyroidal tumors or its restoration in undifferentiated thyroid malignancies. NIS has been transferred into carcinoma cell lines derived from cervix cancer (108), breast cancer (108, 109), glioma (110), hepatoma (111), prostate carcinoma (112–114), non-small cell lung cancer (115), follicular thyroid carcinoma (116), myeloma (117), ovarian cancer (118), and pancreatic cancer (119). Table 1 summarizes all these studies, which have been extensively reviewed elsewhere (120, 121).

However, enabling cells to concentrate radioiodide is not sufficient. Radioiodide must be retained long enough to exert its cytotoxic effect, and the real challenge is to overcome the lack of therapeutic efficacy of accumulated radioiodide in cancer cells. This can be apparently attributed to the rapid radioisotope efflux observed. The radiation dose necessary to have a discernible therapeutic effect is not achieved because of the insufficient retention time of radioiodide ($^{131}I^-$) in the tumor (a few hours) in contrast with the long half-life (8 days) of this isotope. Several approaches have been proposed to overcome this problem. Boland *et al.* used modified vectors and/or higher viral doses to improve the efficiency, yet this does not seem to be plausible for therapeutic purposes because of toxicity (108). Contradictory data concerning lithium salts, increasing the effective half-life of radioiodide in one study (122) but not in another (111), points to the need of more studies to confirm further the practical use of this pharmacological approach. For their part, Smit *et al.* decided to modify iodide environmental conditions of nude mice by using a low-iodide diet and thyroid ablation (116). The short half-life of iodide in NIS-transfected tumors could only be partially improved under these conditions. Stimulating the organification process is another approach aimed at expanding the retention time of iodide inside the cell. In fact, many authors are skeptical about *NIS* gene transfer into nonthyroidal tumors as these are unable to organify and, thus, retain iodide. As has been explained before, the thyroid is the only tissue known to organify iodide to a significant extent. Iodide organification is the covalent incorporation of iodide into selected tyrosyl residues of the Tg molecule and, thus, causes radioiodide to be retained within the gland for several days. TPO is the enzyme responsible for iodide organification. Huang *et al.* cotransfected non-small cell lung cancer cells with

Table 1 Main studies based on *NIS* gene therapy.

Author	Year	Tumor cell lines	Observations
Shimura *et al.*	1997	Transformed rat thyroid cells (FRTL-Tc)	Use of eukaryotic expression vector under CMV promoter. Lack of retention, low absorbed dose and tumor unresponsiveness
Mandell *et al.*	1999	Melanoma (A375), liver (BNL.1 ME), colon (CT26), ovarian (IGROV)	Use of a retroviral vector under SV40 promoter. Only imaging.
Cho *et al.*	2000	Glioma (F98)	Use of retroviral vector under LTR promoter. Intracerebral brain tumor model.
Boland *et al.*	2000	Cervix (SiHa), prostate (DU 145 and PC-3), breast (MCF-7 T-47D), lung (A549), and colon cancer (HT-29)	Use of Ad. vector under CMV promoter. Intratumoral inyection *in vivo.* Only imaging.
Nakamoto *et al.*	2000	Breast (MCF-7)	Use of eukaryotic expression vector. Only imaging
Spitzweg *et al.*	2000	Prostate adenocarcinoma (LNCaP)	Use of eukaryotic expression vector under prostate-specific antigen (PSA) promoter.
Groot-Wassink *et al.*	2002	Human pancreatic adenocarcinoma (HPAF)	Ad. vector under CMV promoter. Positron emission tomography (PET) imaging using ^{124}I
Smit *et al.*	2002	Follicular thyroid carcinoma (FTC 133)	Eukaryotic expression vector under CMV promoter. Radioiodine kinetics improved by conventional conditioning with thyroid ablation and low-iodide diet
Sieger *et al.*	2003	Hepatoma (MH3924A)	Use of the retroviral vector under glucose transporter 1 gene (GTI-1.3) promoter
Haberkon *et al.*	2003	Rat prostate adenocarcinoma (Dunning R3327subline AT1)	Retroviral vector under the elongation factor 1α (EF1-α) promoter. Associated with lack of retention, low absorbed dose and tumor unresponsiveness.
Dingli *et al.*	2003	Myeloma (KAS-6/1 and MM1).	Use of self-inactivating (SIN) lentiviral vector under minimal immunoglobulin promoter
Faivre *et al.*	2004	Model of hepatocarcinoma induced by diethylnitrosamine in immunocompetent Wistar rats	Ad. vector under CMV promoter injected in the portal vein
Shen *et al.*	2004	Glioma (F98)	Rhenium, a potent β ($-$) emitter, is more efficient than ^{131}I to enhance survival.
Dwyer *et al.*	2005	Breast cancer (MDA–MB-231)	Ad. vector under MUC1 promoter. Intratumoral inyection *in vivo*
Dwyer *et al.*	2005	Preclinical large animal model (dogs) of locally recurrent prostate cancer	Direct intraprostatic injection of Ad. vector under de CMV promoter.
Cengic *et al.*	2005	Medullary thyroid carcinoma (TT)	Use of calcitonin promoter. Only *in vitro* study.
Scholz *et al.*	2005	Colon carcinoma (HCT 116)	Use of carcinoembryonic antigen (CEA) promoter. Only *in vitro* study
Dwyer *et al.*	2006	Ovarian cancer (OVCAR-3)	Use of CMV promoter better than MUC1 promoter. Intratumoral inyection *in vivo*
Dwyer *et al.*	2006	Pancreatic cancer (Capan 2)	Ad. vector under MUC1 promoter. Intratumoral inyection *in vivo*

CMV, cytomegalovirus; LTR, long terminal repeat; Ad., adenoviral.

human *NIS* and *TPO* genes. This strategy led to increases in radioiodide uptake and retention, and proved promising (115). By contrast, Boland *et al.* did not obtain a significant increase in the iodide retention time in SiHa human cervix tumor cells, even though the production of an enzymatically active TPO was tested and a significant increase in iodide organification was observed in the targeted cells (123). Furuya *et al.* transferred both TITF-1 and NIS using adenoviral vectors into poorly differentiated human thyroid cells and achieved significantly induced iodide retention and organification in tumors formed in nude mice. Transfection of TITF-1 alone induced reexpression of TPO and Tg, but not NIS expression (124).

That iodide organification is a prerequisite for radioiodide retention is quite arguable, since several groups have recently demonstrated good responses to radioiodide therapy in tumor cells lacking organification. The use of tissue-specific promoters appears to be an important breakthrough in order to improve the overall outcomes in the current studies. Spitzweg *et al.* obtained excellent results by stably transfecting the human prostate adenocarcinoma cell line LNCaP with *NIS* cDNA under the control of the prostate-specific antigen (PSA) promoter. However, the authors used a dose of 3 mCi of $^{131}I^{-}$ per mouse to achieve tumor reduction, higher than the doses used in the previous studies (114). A lower dose (1 mCi) was used by the same group in NIS-transfected myeloma in nude mice based on biodistribution results. They used an immunoglobulin promoter and enhancer elements in a self-inactivating (SIN) lentiviral vector leading to a specific and high-level

transgene expression in myeloma cells. Myeloma is a very radiosensitive malignancy and, concordantly, a complete eradication of tumor xenografts was observed. Furthermore, the authors studied the percentage of nontransduced cells and all of them were killed by electrons emitted by $^{131}I^-$ from distant cells, the so-called 'bystander effect' (125). The same group also found a therapeutic effect of $^{131}I^-$ in colon carcinoma and medullary thyroid carcinoma cell lines following induction of tumor-specific iodide uptake activity by CEA and calcitonin promoter-directed NIS expression respectively, although these studies were only carried out *in vitro* (126). Another promoter of the *MUC1* gene (encoding a transmembrane glycoprotein) seems to be more versatile, as it is overexpressed in many tumor types, including breast, pancreatic and ovarian tumors. Indeed, a 58-fold increase in iodide uptake using $^{123}I^-$, and an 83% reduction in tumor volume after a therapeutic dose of $^{131}I^-$ was observed in infected MUC1/NIS-positive breast cancer tumor xenografts (109). In pancreatic tumor xenografts, administration of therapeutic doses of $^{131}I^-$ resulted in significant regression of MUC1/NIS transduced tumors, with a mean 50% reduction in volume within 10 weeks of therapy. Interestingly, intravenous injection of Ad5/CMV/NIS resulted in robust iodide uptake throughout mouse liver, whereas no uptake was detected in the liver of animals given Ad5/MUC1/NIS intravenously (119). However, in ovarian cancer xenografts, $^{123}I^-$ imaging revealed a clearly less intense image and a lack of tumor reduction after a therapeutic dose of $^{131}I^-$ in MUC1/NIS-transduced tumors. By contrast, a 53% reduction in tumor volume was seen in CMV/NIS-transduced tumors (118).

A surprising study by Faivre *et al*. has provided new insights, as they used an animal model completely different from the conventional tumor-xenografted nude mice. They used an aggressive model of hepatocarcinoma induced by diethylnitrosamine in immunocompetent Wistar rats. After portal vein injection of an adenoviral vector containing the cytomegalovirus promoter (Ad-CMV-rNIS), they obtained marked and sustained (>11 days) iodide uptake as well as strong inhibition of tumor growth and prolonged survival of hepatocarcinoma-bearing rats after radioiodide treatment. Although they observed a massive iodide efflux *in vitro* in human hepatic tumor cell lines, the sustained iodide uptake *in vivo* was attributed to permanent recycling of the effluent radioiodide via the high hepatic blood flow, and not to an active retention mechanism. They supported this idea *in vivo* through the specific inhibition of NIS by sodium perchlorate that led to a rapid iodide efflux from the liver (127). Another promising study by Dwyer and coworkers demonstrated the successful introduction of localized and functional NIS expression in the prostate gland of dogs with locally recurrent prostate cancer using adenovirus-mediated NIS expression under the control of the CMV promoter. No vector-related toxicity was observed, providing further evidence of the safety and efficacy of *NIS* as a therapeutic gene. This study was carried out in preparation for a phase I clinical trial for prostate cancer (113).

Finally, positron emission tomography (PET), a high-resolution imaging modality useful for *in vivo* imaging, can be used in NIS-transduced tumors using ^{124}I, an isotope that emits positrons. PET combined with computerized tomography has a ten times higher resolution than conventional imaging and permits precise anatomical localization. In an attempt to develop this promising approach, two studies have demonstrated that hNIS expression in tumor xenograft models can be monitored by PET after intravenous injection of ^{124}I, demonstrating the potential of this approach for noninvasive imaging (128, 129).

Overall, compared with other genes used in gene therapy, *NIS* offers the unique advantage that it can be used both as a reporter and as a therapeutic gene. *NIS* gene transfer makes it possible to image, monitor and treat the tumor with radioiodide, just as in differentiated thyroid cancer.

Alternative NIS-transported radioisotopes

Rhenium (Re) is a chemical analog of Technetium (Tc) and chemical and biodistribution properties are practically identical to those of Tc. Dadachova *et al.* have recently proposed the use of ^{188}Re for the treatment of NIS-expressing tumors (130). ^{188}Re is a powerful β-emitting radionuclide with a 16.7-h half-life, which is effective over a higher tissue range (23–32 mm), meaning that ^{188}Re can be used for treatment of relatively large tumors. Dosimetry calculations carried out by the same group indicate that in the absence of organification, ^{188}Re-perrhenate delivers a 4.5-fold higher dose to a 2 g tumor in a human than ^{131}I. Encouraging therapy results have been reported in hNIS-transfected hepatocarcinoma cells (131) and in hNIS-expressing glioma tumors in rats (132).

Astatine (^{211}At) is an α-emitting radiohalide with a very high-linear energy, which makes its relative biological efficiency close to maximal, and a short tissue range (60 μm). Several reports have tested this radioisotope in NIS-expressing cancer cell lines (133–135). However, its scarce availability and nontrivial safety issues in production and handling makes this alternative NIS-transported radioisotope unsuitable at present.

Conclusion

As discussed throughout this article, NIS research extends from detailed molecular characterization and

elucidation of regulatory processes at several levels of the follicular thyroid cell, to novel medical applications in human malignancies. NIS has turned out to be a versatile molecule that is expressed and regulated differently in several extra thyroidal tissues, including the breast – and this can be effectively transferred to a wide variety of cancers, from prostate to glioma that do not express NIS endogenously, providing the basis for a novel cytoreductive gene therapy strategy.

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