INTRODUCTION
The current treatment for metastatic papillary and follicular thyroid carcinomas, consists of total thyroidectomy followed by administration of radioiodide for the ablation of any remaining thyroid cancer cells or metastases (1). Radioiodide treatment of thyroid cancer has been employed for over 60 years (2), and this is the most effective targeted and curative radiotherapeutic modality available for any cancer. Radioiodide also destroys any remaining normal thyroid tissue, thus increasing the sensitivity of subsequent $^{131}$I scanning and serum Tg measurements for the detection of recurrent or metastatizing disease. This is because, if normal thyroid cells remained after thyroidectomy, they would tend to prevent cancerous cells from being detected by either method. The success rate of this treatment is impressive: the mortality of patients with metastatic thyroid cancer who are treated with $^{131}$I is just 3%, as opposed to 12% for those who are not treated (3). Side-effects resulting from this therapy, such as mild sialadenitis, are minimal (3); in most instances they resolve within a few weeks of termination of the treatment.

Two key characteristics of the thyroid contribute to the success of this approach. First, thyrocytes, both normal and cancerous, exhibit a remarkable ability to actively transport iodide ($^{1-}$). Thus, when radioiodide is administered, it is actively taken up almost exclusively by thyrocytes without affecting other cells. This makes radioiodide therapy a distinctively specific targeted method that delivers radiation from within the cancerous cells themselves. Even though $^{1-}$ transport activity is significantly lower in the
majority of cancerous thyrocytes than in normal ones, the activity remains high enough for accumulated radioiodide to destroy cancerous thyrocytes as well. The decreased ability of cancerous thyrocytes results in the presence of “cold nodules” at tumor sites on thyroid scintigraphic scans. Second, although the thyroid is physiologically crucial, its function can be fully restored after thyroidectomy by thyroid hormone substitutive therapy, thus keeping patients in a euthyroid state.

It has long been well known that active I⁻ transport is a key attribute of differentiated thyrocytes, as I⁻ is essential for thyroid hormone biosynthesis. The Na⁺/I⁻ symporter (NIS) is the plasma membrane glycoprotein that mediates active I⁻ transport from the bloodstream into the cytoplasm of thyrocytes. Using expression cloning in Xenopus laevis oocytes, our group isolated the cDNA encoding NIS from rat-thyroid-derived FRTL-5 cells (4). On the basis of a high degree of homology with the rat NIS cDNA, the human, mouse, and pig NIS cDNAs were subsequently cloned (5, 6, 7). NIS-mediated active I⁻ transport has also been documented in a few other tissues, including salivary glands, gastric mucosa, and lactating (but not non-lactating) mammary gland (8, 9). These findings and the generation of high affinity anti-NIS Abs have led to a thorough molecular characterization of NIS (10) (11) and to the analysis of both thyroidal and extrathyroidal I⁻ transport in health and disease (9, 12–14).

**FUNCTION AND STRUCTURE OF NIS**

NIS couples the inward translocation of Na⁺ down its electrochemical gradient to the simultaneous inward “uphill” translocation of I⁻ against its electrochemical gradient. NIS activity is inhibited by the “classic” competitive inhibitors perchlorate and thiocyanate (9, 15–18). Two Na⁺ are transported per each I⁻ (19). The Na⁺ gradient that provides the driving force for I⁻ uptake is maintained by the Na⁺/K⁺ ATPase. In the thyroid, both NIS and the Na⁺/K⁺ ATPase are located on the basolateral side of the thyroid follicular cells, facing the blood supply (20). Rat NIS (rNIS) is a 618-amino acid protein (relative molecular mass 65,196) (4); both human and pig NIS, which contain 643 amino acids each, are highly homologous (75.9% and 74.2%, respectively) to rNIS (6, 7). Based on extensive experimental testing, we have proposed a NIS secondary structure model with 13 transmembrane segments (Figure 1) (12). The amino and carboxy termini face extra- and intracellularly, respectively (10). NIS is a glycoprotein; three of its Asp residues (225, 485, 497) are glycosylated in the endoplasmic reticulum (21). However, glycosylation is not essential for proper NIS function, as indicated by the observation that a non-glycosylated NIS protein is properly targeted to the plasma membrane and displays I⁻ transport activity with an identical Kₘ value (~2–30 µM) to that of wild-type (WT) NIS (21). The ca 70-amino acid hydrophilic carboxy terminus is the main phosphorylated region of the protein (22). Freeze-fracture electron microscopy studies of NIS-expressing Xenopus laevis oocytes revealed the appearance of 9-nm intramembrane particles corresponding to NIS (19). The size of these particles suggested that NIS may function as a multimeric protein. Recent co-immunoprecipitation experiments indicate that NIS is indeed an oligomer (23). A putative leucine zipper motif constituted by leucines at positions 199, 206, 213 and 220 may be the structural basis for NIS oligomerization (4).
Figure 1. Iodide transport and biosynthetic pathway of thyroid hormones T₃ and T₄ in the thyroid follicular cell. Thyroid follicles are comprised of a layer of epithelial cells surrounding the colloid. The basolateral surface of the cell is shown on the left side of the figure, and the apical surface on the right. Active accumulation of I⁻, mediated by the Na⁺/I⁻ symporter (NIS) [top circle], driven by the Na⁺ gradient generated by the Na⁺/K⁺ ATPase [bottom circle]; once I⁻ effluxes towards the colloid [cylinder], (TPO) [triangle] catalyzes the organification of I⁻ on the thyroglobulin (Tg) molecule. Dotted line pointing from the apical to the basolateral surface indicates endocytosis of iodinated Tg, followed by its phagolysosomal hydrolysis and secretion of thyroid hormones.

Secondary structure model of NIS. Transmembrane segments are numbered with Roman numerals I-XIII. The N-terminus faces the extracellular milieu and the C-terminus the cytosol. N-glycosylation sites are indicated by arrows and the leucine zipper motif in the VI transmembrane segment is shaded gray. Serines on the C-terminus are indicated.

NIS EXPRESSION IN THYROID CANCER

Given that most thyroid cancers exhibit decreased or absent radioiodide accumulation, the prevailing expectation for a long time was that NIS expression would be found to be decreased or absent in cancerous thyrocytes. The first investigations addressing this issue, carried out using RT-PCR and showing lower mRNA levels in cancerous than in normal thyrocytes, seemed to confirm these expectations (24–28). RT-PCR is an easy-to-perform and very effective technique to detect mRNA expression even in very small tissue samples. However, determinations of mRNA levels by either RT-PCR or
Northern blot analysis provide no information on RNA stability. In addition, mRNA levels of proteins like NIS, with long half-lives and complex posttranscriptional regulation, do not necessarily correlate with actual protein expression (29). Immunoblot analyses to directly assess protein expression would address this limitation; however, this requires significantly larger tissue samples, which are not often available from human specimens.

Immunoblot analyses may provide satisfactory quantitative and qualitative information on NIS protein expression and some posttranslational modifications, but not on subcellular distribution. The subcellular localization of NIS is particularly significant because, as pointed out earlier, NIS is functional only when it is properly targeted to the plasma membrane. Hence, immunohistochemical analysis of NIS expression in thyroid cancer was carried out (20, 30, 31). In addition to revealing the subcellular distribution of NIS, immunohistochemistry offers the advantage that NIS protein expression in the carcinomatous tissue can be compared to the surrounding normal tissue in the same thyroid gland. Surprisingly, immunohistochemical studies of NIS protein expression in thyroid cancer have shown that as many as 70% of thyroid cancers actually exhibit NIS protein overexpression (Figure 2B), as compared to the surrounding normal tissue, although in these cancerous cells NIS is mainly located in intracellular membrane compartments rather than in the plasma membrane. NIS was absent only in about 30% of the cases (20, 31). Thus far, no NIS mutations resulting in impaired protein expression or altered plasma membrane trafficking have been identified in thyroid cancer (32). These findings have had a significant impact on research approaches aiming to improve the effectiveness of radioiodide therapy, since they emphasize the importance of stimulating NIS targeting to and/or retention at the plasma membrane rather than stimulating NIS expression at the transcriptional level.

UNDERSTANDING NIS REGULATION IN HEALTH AND DISEASE MAY IMPROVE THE EFFECTIVENESS OF RADIOIODIDE TREATMENT

TSH and \( I^- \) are the two main factors that regulate NIS expression: TSH stimulates and \( I^- \) decreases it. Hence, TSH stimulation and \( I^- \) depletion of residual thyroid carcinoma tissue are the two most important modulators routinely used to optimize radioiodide treatment. To achieve maximum therapeutic effect, thyroidectomized patients must have TSH levels above 30 mU/l and must have been on a low \( I^- \) diet for two weeks prior to initiation of radioiodide treatment (1).

TSH has long been known to be a key regulator not only of NIS expression but also of thyroidal \( I^- \) uptake (i.e., NIS activity). No thyroidal NIS expression is observed in hypophysectomized rats (because of the lack of TSH), but thyroidal NIS expression is restored as early as 24 h after treatment with TSH. In intact (i.e., non-hypophysectomized) rats, treatment with the \( I^- \) organification inhibitor propylthiouracil causes elevated TSH levels, which in turn lead to higher NIS expression than in control animals (10). TSH regulates NIS expression at both the transcriptional and posttranscriptional levels. Several groups have demonstrated that TSH upregulates \( I^- \) transport by a cAMP-mediated increase in NIS transcription, while withdrawing TSH causes decreased cAMP levels and diminished NIS transcription (33).
Figure 2. A: NIS immunohistochemistry in Graves’ disease. NIS is localized in the basolateral plasmamembrane B: NIS immunohistochemistry in follicular carcinoma shows the intracellular localization of the significantly overexpressed NIS protein C: Indirect immunofluorescence analysis of NIS localized in the plasma membrane of FRTL-5 cells kept in the presence of TSH. D: intracellular NIS localization in TSH deprived FRTL-5 cells E: schematic representation of NIS plasma membrane localization and iodide transport in FRTL-5 cells kept in the presence of TSH F: schematic representation of NIS localized in the intracellular membrane compartments in TSH deprived FRTL-5 cells, resulting in lack of iodide transport.

The detailed analysis of the rat and human NIS promoters has confirmed the significant role of Pax8 in NIS expression (34–36). In rat, the proximal NIS promoter was found to contain a TTF1 binding site and a TSH-responsive element where a putative transcription factor NTF-1 (NIS TSH-responsive factor 1) interacts (39). NIS upstream enhancer (NUE-2495 to -2260) contains two Pax-8 binding sites and a degenerate CRE (cAMP-responsive element sequence), which are essential for full TSH cAMP-dependent transcription of NIS (34). Interestingly, during chronic TSH stimulation when the catalytic subunit of PKA is downregulated, cAMP is still able to stimulate NIS transcription, indicating the existence of both PKA-dependent and independent mechanisms (34). Recently, a thyroid-specific far-upstream (−9847 to −8968) enhancer in the human NIS gene – highly homologous to the rat NUE – has been reported. It contains putative Pax-8 and TTF-1 binding sites and a CRE-like sequence. The TTF-1 binding site is not required for full activity (35, 36).
FRTL-5 cells are rat-thyroid-derived, well-differentiated normal thyroid epithelial cells that grow in media supplemented with TSH. These cells are frequently used as an in vitro model system to study TSH regulation. In FRTL-5 cells, NIS expression is TSH dependent. Kaminsky et al. (37) observed that, in the absence of TSH in the medium, intact FRTL-5 cells did not transport $\Gamma^-$, whereas membrane vesicles prepared from the same TSH-deprived cells surprisingly maintained their $\Gamma^-$ transporting ability. This suggested that mechanisms other than transcriptional might also operate in regulating NIS activity in response to TSH. Riedel et al. (22) investigated this phenomenon in detail. They observed that in the absence of TSH, there was no de novo NIS synthesis in FRTL-5 cells, while previously synthesized NIS was redistributed from the plasma membrane to intracellular membrane compartments. (Figure 2 C,D,E,F) These authors also demonstrated that NIS has a long half-life: 5 days in the presence and 3 days in the absence of TSH. Considering the TSH regulation of NIS expression and the long half-life of NIS, it is clear that NIS mRNA levels alone are not a good indicator of actual NIS protein levels. Instead, NIS protein levels must be assessed directly with anti-NIS Abs. In addition, it is also essential to keep in mind that NIS protein expression, in turn, does not necessarily correlate with NIS activity, because such factors as subcellular distribution of NIS to the plasma membrane play a key role in NIS function; hence, it is crucial to quantitate NIS activity (Figure 3).

**TSH modulates NIS phosphorylation**

The mechanism by which TSH regulates the subcellular distribution of NIS is unknown. Phosphorylation has been shown to be implicated in the activation and
subcellular distribution of several transporters (38–40). NIS has several consensus sites for kinases, including those for cAMP-dependent protein kinase, protein kinase C, and casein kinase-2 (9, 22). Furthermore, TSH actions in the thyroid are mainly mediated by cAMP. All these points raised the possibility that phosphorylation might be involved in the regulation of NIS subcellular distribution. When FRTL-5 cells were labeled with $^{32}$P$_i$, lysed, and immunoprecipitated with anti-NIS Ab, it was observed in the autoradiogram that NIS was phosphorylated, independently of the presence of TSH in the culture medium (22). The phosphopeptide map obtained after NIS digestion with trypsin was markedly different when TSH was present from that when TSH was absent (22).

The predominant phosphorylated region of NIS was determined by treatment of the immunoprecipitated symporter with CNBr. CNBr cleaves polypeptides at methionine residues. The anti-NIS Ab generated against the last 16 amino acids of NIS recognized an 11-kDa polypeptide observed also by autoradiography. The densitometric quantitation of the autoradiogram indicated that the major phosphorylation region of NIS is the carboxy terminus (22). Moreover, TSH increased the phosphorylation level of the COOH terminus of NIS ~16-fold. For the identification of which of the serine residues within the COOH terminus are phosphorylated, S551, S552, S568, and S581 (Fig. 1) were replaced individually and simultaneously with alanine. Significantly, the replacement of the four serines of the COOH terminus promoted phosphorylation of threonines in NIS, suggesting that there is an important biological pressure to preserve phosphorylation of NIS at the COOH terminus (22). Future experiments should elucidate whether NIS phosphorylation is involved in trafficking and/or retention of NIS at the plasma membrane.

**Regulation of NIS expression by I$^-$_**

As indicated above, I$^-$ itself, the substrate of NIS, also regulates NIS expression, but the mechanism of this regulation is less clear than that of TSH. For over 60 years, it has been known that I$^-$ organification and, consequently, thyroid hormone biosynthesis, are blocked when the intracellular concentration of I$^-$ rises to a certain threshold. This phenomenon (i.e., the inhibition of I$^-$ organification by a high concentration of I$^-$) is called the Wolff-Chaikoff effect, and it has been used to block thyroid function in hyperthyroid patients (41, 42). I$^-$ also suppresses I$^-$_ transport in a time- and dose-dependent manner, an effect that has been investigated by several groups both in vitro and in vivo (43–45). As I$^-$ transport decreases, the intrathyroidal I$^-$ concentration falls, the inhibition of organification is relieved, and thyroid hormone synthesis resumes; thus, by downregulating its own I$^-$_ transport, the thyroid “escapes” from the inhibitory effect of I$^-$ overload. Grollmann et al. (45) investigated the effect of I$^-$ preincubation on I$^-$ uptake in FRTL-5 cells, and found that I$^-$ preincubation of these cells suppresses I$^-$_ uptake in a dose- and time-dependent manner. These authors observed decreased I$^-$_ transport after a 2-h incubation with 100 μM of NaI (45).

With the availability of the NIS cDNA and anti-NIS Abs, the inhibitory effect of I$^-$ on its own transport in the thyroid has been partially reinvestigated by several groups. In dog, Uytterspot et al. (46) found that I$^-$ inhibited both TPO and NIS
mRNA expression, but no protein levels were measured. In FRTL-5 cells, Spitzweg et al (47) and Eng et al (48) published somewhat contradictory results. Spitzweg et al (47) reported a 50% decrease in $I^-$ uptake. However, in these uptake studies, the specific activity of the radioactive $I^-$ used in the transport measurements was diluted out by preincubation with unlabeled $I^-$, which results in its intracellular accumulation. Without taking this factor into account, the interpretation of these findings is uncertain. These authors also reported a decrease in NIS mRNA levels, but did not determine NIS protein. In contrast, Eng et al. (48) did not find decreased NIS mRNA levels after $I^-$ preincubation. Instead, they found that both the levels and the half-life of the NIS protein were significantly decreased. Hence, Eng et al. (48) concluded that $I^-$ regulates its own transport in FRTL-5 cells mostly by posttranscriptional mechanisms. Surprisingly, the same authors found that both NIS mRNA and protein levels were decreased in vivo in response to the administration of $I^-$ (49). Evidently, a thorough molecular examination of the regulatory effect of $I^-$ on $I^-$ transport simultaneously assessing NIS expression, subcellular localization, and kinetic properties is required to understand the intriguing role of $I^-$ in its own transport.

**Effect of spatial organization of thyroid cells**

It is clear from earlier studies that the spatial organization and apical-basolateral polarization of thyroid epithelial cells significantly influence their functions, including $I^-$ transport, $I^-$ organification, and protein expression. Roger et al (50) isolated human thyroid epithelial cells from normal subjects and grew the cells in the presence or absence of serum. They observed that, whereas cells grown in the presence of serum formed monolayers, in the absence of serum the cells formed aggregates. Following TSH stimulation, cells in aggregates exhibited more avid $I^-$ transport than cells in monolayers. Interestingly, this TSH stimulatory effect was abolished by the addition of serum to the medium. Takasu et al (51) showed that, in porcine thyroid cells, polarity is important for $I^-$ uptake, and a follicular structure is required for $I^-$ organification. Kogai et al (52) have recently reinvestigated the effect of spatial organization of thyrocytes on NIS expression and function. They showed that TSH upregulates both NIS mRNA and protein levels in 2- and 3-dimensional human thyrocyte primary cultures, but a significant increase in $I^-$ uptake occurs only in 3-dimensional structures.

**SPECIFIC CONSIDERATIONS RELATED TO RADIOIODIDE TREATMENT**

A prerequisite for the success of radioiodide treatment is the retention of the radioisotope for a sufficiently long time so the necessary dose is delivered to destroy the malignant tissue. The retention time of radioiodide in thyrocytes is determined by $I^-$ uptake and $I^-$ efflux. At steady-state conditions, $I^-$ accumulation reflects the equilibrium between the rates of influx and efflux (Figure 4).

In the healthy thyroid gland, NIS mediates the active accumulation of $I^-$, whereas the mechanisms involved in $I^-$ efflux are poorly understood (see “$I^-$ efflux: pendrin and AIT” below). $I^-$ organification – i.e., the TPO-mediated iodination of the tyrosine residues on the thyroglobulin molecule – occurs on the colloidal surface of the apical membrane. Iodinated thyroglobulin molecules remain in the colloid, surrounded
by the thyroid epithelial cells, thus increasing the radioiodide retention time in the thyroid gland. In contrast, in thyroid cancer, the typical follicular architecture of normal thyroid tissue is not conserved, as the malignantly transformed epithelial cells lose their polarity (53). Hence, these cells display no well-defined colloidal space, and as a result, thyroglobulin leaks out into the extracellular space and the bloodstream. Most differentiated thyroid cancers exhibit TPO protein expression, but at levels lower than those considered normal (54–56); TPO gene mutations have also been reported in some differentiated thyroid carcinomas (57). Furthermore, earlier studies in humans showed impaired or absent I⁻ organization in thyroid cancer (58), underscoring the loss or reduction of organification in thyroid cancer.

More recently, the organification effect in radioiodide retention time has been assessed in vivo in non-NIS-expressing tumors into which both rat and human NIS have been introduced under the control of tissue-specific promoters. Since these tumors do not express TPO, they do not organify I⁻. Cho et al (59) reported radioiodide retention time greater than 24 h in hNIS-expressing xenografted human glioma cells in rats, and observed a longer survival in animals with NIS-expressing tumors versus control animals with non-NIS-expressing tumors. Spitzweg et al (60) introduced NIS in a recombinant adenovirus into a human prostate carcinoma cell line under the regulation of the prostate specific antigen (PSA) promoter. They reported a retention time of 5.6+/−1.4 h in NIS-expressing prostate carcinoma xenografts in nude mice and a remarkable decrease (over 80%) of the size of these xenografts after a single ip injection of 3 mCi $^{131}$I. Dingli and colleges (61), for their part, expressed NIS in a myeloma cell line using a transcriptionally targeted lentiviral vector, where the therapeutic or reporter

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**Figure 4.** Determinants of iodide accumulation.
Thyroidal iodide transport and thyroid cancer

Gene is under the control of minimal immunoglobulin promoter and enhancer elements (immunoglobulin κ-light chain enhancer elements). These authors also investigated the so-called bystander effect. β-particles emitted during the decay of $^{131}$I can travel a distance of 0.2–2.4 mm. Therefore, the isotope is capable of destroying "bystanding" non-NIS-expressing cells. Dingli et al. (61) also treated myeloma xenografts containing variable numbers of radioiodide-transporting, NIS- and non-NIS-transduced tumor cells. The result was striking: all tumors in which 50–100% of the cells expressed NIS had completely regressed two weeks after a single dose of 1 mCi $^{131}$I.

The above results provide strong evidence against the widely held notion that radioiodide therapy is likely to be ineffective in non-thyroidal cells that, while functionally expressing NIS (whether endogenously or by targeted transfection), lack the ability to organify. The reasoning was that the absence of organification resulted in the isotope not being retained in the cells for a sufficiently long time. Yet, in the mentioned studies (59, 61, 62), radioiodide treatment was effective even in the absence of I$^{-}$ organification.

If organification is not essential for radioiodide therapy to be effective, sufficient iodide uptake mediated by NIS and slow I$^{-}$ efflux are the requirements for successful radioiodide therapy.

I$^{-}$ efflux: pendrin and AIT

Several groups have tried to identify the mediator of apical I$^{-}$ efflux in thyroid epithelial cells. Two recently cloned molecules are the main candidates: pendrin and the apical iodide transporter (AIT) (Figure 1).

In 1997, a gene defective in Pendred syndrome (PDS) was identified by positional cloning (63). Pendred syndrome is characterized by sensorineural (most often prelingual) deafness and goiter with defective organification. In PDS, goiter can develop at any age or may be absent, whereas deafness is generally present (63). Pendrin has been localized on the apical membrane of the thyroid epithelial cells by immunohistochemistry (64). In heterologous expression systems, pendrin has been shown to transport iodide, chloride, formate, and nitrate (65).

The organification defect characteristic of Pendred syndrome was attributed to defective pendrin-mediated apical I$^{-}$ transport into the colloid, where organification occurs (Figure 1). Surprisingly, although the recently generated Pds-knockout mice are completely deaf, they do not exhibit a pathologic thyroidal phenotype (66); therefore, pendrin’s function as the apical I$^{-}$ transporter remains to be further investigated.

By means of a PCR cloning strategy based on NIS-sequence homologies, a 610-amino-acid protein-coding gene was recently cloned from a human kidney cDNA library. The newly identified protein shares both a strikingly high identity (46%) and similarity (70%) to hNIS (67). This protein, called the human apical iodide transporter (hAIT), has been localized to the apical membrane of thyroid epithelial cells; however, a thorough molecular and kinetic characterization is required to unequivocally establish whether hAIT mediates “downhill” movements of I$^{-}$ from the cytosol to the colloid.

AIT (SLC5A8) expression in thyroid carcinomas has not yet been investigated. Interestingly, Li et al (68) found, while screening hypermethylated sequences in colon...
carcinoma cell lines and human colon carcinoma tissues, that the hAIT gene is heavily methylated and hAIT mRNA expression is decreased or absent. Reintroducing AIT into colon cancer cell lines harboring methylated endogenous AIT suppressed their ability to form colonies in soft agar and xenograft tumors in athymic mice. Based on their observations, the authors suggested that AIT could play a role as a tumor suppressor in colon cancer (68).

**STUNNING**

Stunning is the decrease in radioiodide uptake in thyroid tissue caused by previous exposure of the tissue to a tracer dose of the radioisotope for dosimetry. Stunning was first described almost 50 years ago (69), and since then, several clinical studies have been carried out to investigate its deleterious effect on radioiodide therapy. Before treatment with radioiodide, patients usually undergo diagnostic and dosimetric studies. After administering a tracer dose of radioiodide, the percentage of radioiodide uptake in the tumor tissue is determined and used as a basis to calculate the appropriate therapeutic dose. However, stunning is often observed, so that the percentage of radioiodide uptake measured upon administration of the therapeutic dose is significantly lower than that when the tracer dose was first administered for dosimetry. It is hypothesized that stunning is caused by radiation damage to the cells from the previously administered tracer dose. When stunning occurs, the delivered radioiodide therapeutic dose is insufficient for successful ablation of the malignant tissue (70).

While most studies have investigated stunning in vivo during the treatment of patients (71), Postgard et al (72) recently reported an in vitro study revealing more about the possible mechanism of $^{131}$I stunning. These investigators used porcine thyroid cell primary cultures grown on a filter in a bicameral chamber. The apical and basolateral media were separated by the cells assembled into a monolayer. Thyroid cells kept in TSH- and methimazole-supplemented medium were exposed to different amounts of $^{131}$I. Stable iodide (10 nM) was administered to control cells. Transepithelial $^{125}$I transport (from the basal to the apical membrane) was evaluated after a 3-day washout period. Iodide transport decreased 50% with a 3-Gy absorbed dose, and it was almost completely inhibited with an 80-Gy dose. The transepithelial electrical resistance of the cell monolayer was unchanged, showing that the integrity of the epithelial monolayer remained intact. The presence of perchlorate – the competitive inhibitor of I\(^-\) transport – during $^{131}$I incubation, partially prevented the reduction of $^{125}$I transport. Considering that there was no cell damage from radiation exposure and that the same amount of stable iodide had no effect on transport, these authors concluded that decreased I\(^-\) transport after $^{131}$I exposure was most probably the result of a direct effect of radiation on thyroid function.

**IMPROVING I\(^-\) TRANSPORT IN THYROID CANCER**

To achieve an optimal therapeutic effect with maximum radioiodide uptake in thyroid carcinoma metastases, TSH has to be above 30 mU/l in thyroidectomized patients, who must be on a low iodide diet for two weeks prior to treatment (1, 73).
Radioiodide uptake in thyroid cancer could be increased by stimulation of NIS activity. Experimental therapies aiming at restoring NIS function in thyroid cancer have concentrated only on increasing NIS transcription. However, as mentioned earlier, TSH and I\(^-\) are the main regulators of NIS expression and plasma membrane targeting; therefore, high TSH and low I\(^-\) levels are optimal for upregulating NIS expression and cell surface targeting. Clearly, elucidation of the mechanisms involved in NIS targeting to and retention at the plasma membrane may result in novel therapies for thyroid cancer treatment, since NIS is overexpressed in 70% of thyroid cancers but not properly targeted to the plasma membrane (20).

Several groups have attempted to induce NIS transcription in non-NIS-expressing thyroid carcinoma cell lines. Transcriptionally inactive promoter regions often contain hypermethylated CpG rich regions (5-methylcytidine immediately followed by guanidine). These methylated sites of the DNA were found to bind specifically to histone-deacetylase complexes. The N-terminal lysines of unacetylated histones are positively charged and interact with DNA phosphates preventing the binding of transcription factors. When the positive charge of the N-terminus is neutralized via acetylation of the lysines, their electrostatic interaction with the DNA is disrupted, making the binding of transcription factors to the DNA possible. Therefore, inhibiting histone deacetylase activity and/or demethylating CpG-rich promoter regions would initiate transcriptional activity.

As the hNIS promoter has CpG-rich regions, Venkataraman et al. (74) hypothesized that hypermethylation of the hNIS promoter resulting in transcriptional failure could be responsible for decreased or absent NIS expression. These authors were able to restore hNIS mRNA expression in 4 out of 7 cell lines using 5-azacytidine and sodium butyrate treatment. The increase in NIS mRNA transcription correlated with demethylation of the untranslated region in the first exon of the hNIS gene. They also investigated NIS mRNA expression by Northern blot and methylation status of the hNIS promoter in proximity to the TATA box in human thyroid tumors. NIS mRNA expression was observed in 16 out of 22 carcinomas, including papillary, follicular, and anaplastic subtypes. These findings suggest that, in these cases, posttranscriptional mechanisms are probably responsible for decreased I\(^-\) uptake. In the six non-NIS-mRNA-expressing papillary carcinomas, the hNIS promoter was strongly methylated.

Kitazono et al. (75) reported increased NIS mRNA expression detected by Northern blot and quantitative RT-PCR in four human thyroid carcinoma cell lines (two follicular and two anaplastic) \textit{in vitro} after treatment with depsipeptide, a histone deacetylase inhibitor. This increase in NIS mRNA expression was accompanied by an increase in I\(^-\) uptake.

Zarnegar et al. (76) used another histone-deacetylase inhibitor, trichostatin, in papillary, Hurthle, and follicular carcinoma-derived cell lines, and found increased NIS mRNA expression by quantitative PCR. NIS protein levels and I\(^-\) uptake activity were not determined.

Other investigators have tried to achieve redifferentiation of thyroid cancer cells with \textit{trans}-retinoic acid (tRa) treatment in thyroid carcinomas to restore radioiodide uptake. Schmutzler et al (77) were able to upregulate NIS mRNA expression by growing
follicular thyroid carcinoma-derived cell lines in media supplemented with 1 μM tRa for one week, but no effect was observed on either protein expression or I⁻ uptake ability. Surprisingly, the same treatment decreased I⁻ uptake activity in the highly differentiated FRTL-5 cell line (78).

CONCLUDING REMARKS

The role of thyroidal I⁻ transport in the treatment of thyroid cancer is difficult to overestimate. For over 60 years, the administration of radioiodide to thyroid cancer patients after thyroidectomy has been the most effective internal targeted anticancer radiotherapy available, on account of the unique specificity of NIS. Radioiodide therapy is not only effective and specific, it is also remarkably free of severe side effects. This article shows how, upon isolation of the NIS cDNA and the characterization of the NIS molecule, considerable strides have been made in our understanding of NIS regulation at all levels, including biosynthesis, biogenesis, half-life, targeting, and subcellular localization. These advances considerably increase our potential ability to manipulate the system to optimize the effectiveness of radioiodide treatment. In addition, the discovery that NIS is expressed endogenously in breast cancer has raised, for the first time, the realistic prospect of effectively applying radioiodide therapy in extrathyroidal cancers that express NIS endogenously. Finally, recent studies on the transfer of the NIS gene to cancers that otherwise lack endogenous NIS expression, have opened the door to the possible use of radioiodide therapy in these cancers as well.

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