THYROID
Volume 11, Number 5, 2001
Mary Ann Liebert, Inc.

Sodium Iodide Symporter in Health and Disease

Daniel H.Y. Shen,[1] Richard T. Kloos,[2,3] Ernest L. Mazzaferri,[2] and Sissy M. Jhiang[1,2]

Radioiodine-concentrating activity in thyroid tissues has allowed the use of radioiodine as a diagnostic and therapeutic agent for patients with thyroid disorders such as well-differentiated thyroid cancer. However, some extrathyroidal tissues also take up radioiodine, contributing to unwanted side effects of radioiodine therapy. Now that the molecule that mediates radioiodine uptake, the sodium iodide symporter (NIS), has been cloned and characterized, it may be possible to develop novel strategies to differentially modulate NIS expression and/or activity, enhancing it in target tissues and impeding it in others. In addition to restoring NIS expression/activity to ensure sufficient radioiodine uptake for the diagnosis and treatment of advanced thyroid cancers, we envision that it may be possible to selectively increase or confer NIS expression/activity in tumors of nonthyroidal tissues to facilitate the use of radioiodine in their diagnosis and treatment. We also consider the molecular basis of thyroid and nonthyroid disorders that may be complicated by NIS deregulation. Finally, we explore the use of NIS as an imaging reporter gene to monitor the expression profile of the transgene in transgenic mouse animal models and in patients undergoing gene therapy clinical trials.

Introduction

The sodium iodide symporter (NIS) is a membrane glycoprotein that mediates active iodide uptake in the thyroid gland and several extrathyroidal tissues. After molecular cloning of NIS, its structure and function were characterized, and factors affecting its expression/activity have been investigated (for review, see Jhiang [1] and Vieja et al. [2]). NIS is believed to span the plasma membrane 13 times, exhibiting an extracellular NH_2 terminus and a cytosolic COOH terminus. Even though the degree of NIS glycosylation appears to be different among different tissues, there are no studies showing that this alters the function and stability of NIS. Although the exact mechanisms remain to be elucidated, several hydroxy-containing amino acid residues located in transmembrane segment IX (Ser-353, Thr-354, Ser-356, Thr-357) appear to play an important role in NIS activity (2). Electrophysiological analysis of NIS indicates that a 2:1 sodium iodide (Na^+/I^-) stoichiometry, which results in a steady-state inward current, is induced by a net influx of Na^+. Based on kinetic studies, NIS appears to be bound by Na^+ before it is bound by I^-. However, perchlorate, the best-characterized NIS inhibitor, appears to act as a blocker rather than as a competitive substrate.

Because NIS forms the molecular basis of using radioiodine as a scintigraphic imaging and therapeutic agent for tissues showing iodide uptake, it is important to determine how NIS expression/activity is regulated in these tissues. Current investigations generally have taken two different approaches. One is to screen for factors that modulate NIS expression and/or activity, and then to examine the molecular mechanisms by which this occurs. The other approach is to characterize the NIS promoter to identify *cis*-acting elements responsible for NIS regulation, and then to search for the transcription factors that bind them. While controversy exists regarding the characterization of NIS promoter (3,4), it is now generally believed that the NIS minimal promoter alone is insufficient to confer thyroid-specific NIS expression (5–8). Among the *cis*-acting elements identified, an enhancer located between −2,264 and −2,495 base pair (bp) in rat NIS (rNIS), which is located upstream of the rNIS minimal promoter, recapitulates the most relevant aspects of rNIS regulation (8). This rNIS enhancer is sufficient to confer thyroid-specific expression and thyrotropin (TSH)-, and cyclic adenosine monophosphate (cAMP)-stimulated expression of the reporter gene driven by either the NIS minimal promoter or a heterologous promoter. Further understanding of the molecular mechanisms controlling NIS expression and NIS activity in various tissues will likely provide information to improve the clinical management of patients with advanced thyroid cancer, and might create ways to use radioiodine to treat patients with nonthyroid cancer.

Tissue Distribution of NIS

Radioiodine is commonly used to destroy overactive thyroid follicular cells in patients with Graves' disease or toxic nodular goiter, to reduce the size of large euthyroid multi-

Departments of [1]Physiology and Cell Biology, [2]Internal Medicine, and [3]Radiology, The Ohio State University, Columbus, Ohio.

nodular goiters, to ablate normal and malignant thyroid tissues in patients who have undergone total thyroidectomy for papillary or follicular thyroid carcinoma, and to perform whole-body scans to detect recurrent and metastatic thyroid cancers. However, radioiodine uptake, as well as NIS expression, is not restricted to thyroid tissues. Radioiodine uptake by extrathyroidal tissues not only contributes to unwanted side effects of radiation, but it also contributes to difficulty in distinguish incidental findings on diagnostic scintigraphy, such as thymus uptake, from true metastatic thyroid lesions. Therefore, it is of clinical importance to determine the tissue distribution of NIS expression.

Prior to its molecular cloning, the possible tissue distribution and cellular localization of NIS were indicated by studies of radioiodine biodistribution in laboratory animals as well as in human nuclear medicine studies using radioiodine or ^{99m}Tc pertechnetate (a known substrate of NIS) for scintiscans. In addition to thyroid tissues, uptake of radioiodine or ^{99m}Tc pertechnetate is almost always found in the salivary glands, stomach, lactating breast, nasal mucosa, and the placenta (although radioiodine should not be given during pregnancy). However, TSH only stimulates radioiodine uptake in thyroid tissues but not in these extrathyroidal tissues because of their absence of TSH receptors. Occasionally, radioiodine uptake is also seen in the nonlactating breasts of women (9). Radioiodine secretion in tears and the supraorbital uptake of ^{99m}Tc pertechnetate in patients without eye disorders suggest that active iodide uptake might also occur in the lacrimal glands (10). Other extrathyroidal tissues that take up radioiodine are hair follicles (11) and thymus (12,13).

Tissue distribution of NIS mRNA has been investigated by Northern blot analysis, RNase protection assay, and reverse transcription-polymerase chain reaction (RT-PCR). Using NIS antibodies to identify its immunohistochemical cellular localization, NIS protein has been demonstrated in the salivary glands, stomach, lactating breast, lacrimal glands, and thymus. NIS expression is also found by RT-PCR in many other extrathyroidal tissues that are not known to concentrate radioiodine *in vivo* (1,2). Because RT-PCR is a sensitive method that detects low levels of NIS mRNA in a cell population, the clinical significance of NIS expression in these tissues requires further investigation. It is possible that the limited resolution of conventional scintiscans using a gamma camera is too insensitive to detect radioiodine in these tissues. Extrathyroidal tissues that minimally take up radioiodine are more likely to be identified using ^{124}I positron emission tomography (PET). Ideally, the internal radiation dose for nonthyroidal tissues should be estimated to evaluate the possibility of radiation injury when radioiodine is administrated for clinical purposes.

Factors That Modulate NIS Expression

Identifying the factors modulating NIS expression is of both physiological and clinical importance. When radioiodine is given to patients with thyroid diseases, it would be desirable to increase thyroid NIS expression to ensure sufficient uptake for optimal imaging and effective treatment. At the same time, it is important to decrease NIS expression in extrathyroidal tissues to minimize unwanted side effects of radioiodine. Because radioiodine plays a major role in the management of thyroid disease, factors that modulate its uptake and NIS expression have been intensively studied (for review see Jhiang [1]).

Many reagents that modulate iodide uptake have been used in the clinical management of thyroid disorders (Table 1). For example, serum TSH, the primary stimulator of NIS expression in thyroid follicular cells, must be elevated for optimal ^{131}I whole-body scanning and ^{131}I treatment of thyroid cancer. Conversely, iodide directly decreases NIS expression and impairs the organification and release of thyroid hormone, making it therapeutically useful in hyperthyroid patients who require rapid inhibition of thyroxine (T_4) release to prevent thyroid crisis in certain situations such as thyroidectomy.

Many drugs may affect thyroid NIS status by modulating its expression (Table 2). Hydrocortisone, dexamethasone, sex steroids, RU486, amiodarone, bromide, and ketoconazole are known to decrease thyroidal iodide uptake or NIS expres-

Table 1. Modulation of Iodide Uptake Activity and NIS Expression by Reagents Used for Clinical Management of Thyroid Diseases

Reagent	*IUA*	*NIS*	*Clinical relevance*
TSH	↑	↑ [87–89]	To prepare for radioiodine scan, and radioiodine therapy for patients with thyroid cancer.
Iodine	↓	↓ [14,90,91]	To prevent thyroid crisis due to stress such as surgical procedure, and to provide rapid relief of thyrotoxicosis in patients with hyperthyroidism. To block thyroid uptake when using ^{131}I labeled diagnostic and therapeutic compounds and nuclear accidents.
T_3 and T_4	↓	↓ [14]	To prepare for ^{131}I treatment of toxic nodules.
Perchlorate	↓	N [14]	To block unwanted radioiodine uptake, or ^{99m}Tc pertechnetate uptake, in thyroid tissues
TPO inhibitor (MMI, PTU)	N [89]	N [89], ↓ [14]	To provide medical management for patients with hyperthyroidism

IUA, iodide uptake activity; NIS, sodium iodide symporter expression; TSH, thyrotropin; T_3, triiodothyronine; T_4, thyroxine; TPO, thyroperoxidase, MMI, methimazole; PTU, propylthiouracil; ↓, decrease; ↑, increase; N, no change. References are indicated by superscript numbers.

Table 2. Modulation of Iodide Uptake Activity and NIS Expression by Reagents Used for Clinical Management of Nonthyroidal Diseases

Reagent	*IUA*	*NIS*	*Clinical relevance*
Hydrocortisone		↓ [14]	Immunosuppressor
Sex steroids: Progesterone, E_2, DHT	N [15], ↓ [16]	↓ [16]	Sexual hormone replacement therapy
Dexamethasone	↑ [15], ↓ [14]	↓ [14]	Potent anti-inflammatory drug
RU 486 (Mifepristone)	↓ [15]		Arbotifacient or anti-Cushing's drug
Lithium	↓ [92,93]		Antipsychotics
Iodine contrast media	↓ [18]		Radiological examination
Potassium iodide (KI)	↓ [18]	↓ [14]	Nutrition supplements, and contents of some expectorants
Amiodarone	↓ [17,18]		Antiarrhythmic drug
Bromide	↓ [18]		Contents of some expectorants
Econazole	↓ [19]	↓ [19]	Common anti-fungal drug
Adenosine	↑ [20]	↑ [20]	Cardiovascular drug
Retinoic acid	↑ [21–23]	↑ [22,23]	Redifferentiation therapy for a variety of malignancies
Ethanol	↑ [47]		Long-term ethanol consumption in alcoholism
Bile acid	↓ [48]		Bile acid accumulation in chronic active hepatitis patients

IUA, iodide uptake activity; NIS, sodium iodide symporter expression; E_2, estrodiol; DHT, dihydrotesterone; MMI, methimazole; PTU, propylthiouracil; ↓, decrease; ↑, increase; N, no change. References are indicated by superscript numbers.

sion (14–19). Perhaps some of these drugs should be avoided in patients who are to receive radioiodine therapy. In contrast, adenosine and retinoic acid increase thyroidal iodide uptake and NIS expression (20–22). Understanding the molecular mechanisms underlying these two reagents' stimulatory effects on NIS expression might lead to the development of novel strategies to increase the effectiveness of radioiodine treatment (for review see Schmutzler and Koehrle [23]). Several cytokines and growth factors alter thyroid hormone secretion and metabolism, perhaps due to their modulating effects on NIS expression (24–31). These cytokines and growth factors, which may be administered for therapeutic purpose, might also originate from a thyroid disorder, from non-thyroid illness, or the aging process (31–35).

NIS and Thyroid Diseases

The physiological significance of NIS is best demonstrated in patients with an iodide transport defect (ITD) who have germline mutation(s) in both NIS alleles. Patients with ITD present with congenital hypothyroidism with or without goiter, but with no clinical symptoms from extrathyroidal tissues that express nonfunctional NIS. Thus it appears that NIS does not play an important physiological role in extrathyroidal tissues such as the salivary gland, stomach, and the nonlactating breast.

Patients with ITD can be identified by their absence of radioiodine or ^{99m}Tc pertechnetate uptake in both thyroid and salivary glands. Nonetheless, few patients with ITD are diagnosed, probably because congenital hypothyroidism is so easily treated with levothyroxine without determining the etiology. Among the few ITD patients identified, germline NIS mutations have been identified (for review see Vieja et al. [2] and Pohlenz and Refetoff [36]). Perhaps due to its low incidence, there has been little clinical interest in performing genetic testing for ITD among patients with congenital hypothyroidism. It is nonetheless important to identify ITD among children with congenital hypothyroidism so their affected siblings who may not yet be hypothyroid can be identified. Patients with ITD living in areas rich in dietary iodine may not develop hypothyroidism until later in life and thus may not be diagnosed at birth by screening tests for congenital hypothyroidism. Because ITD may be caused by a variety of NIS mutations, it is diagnostically expedient to simply perform a ^{99m}Tc pertechnetate scan in children with congenital hypothyroidism to identify those who have an absence of both thyroid and salivary uptake (37). After ITD is diagnosed in an index case, affected family members can then be identified by ^{99m}Tc pertechnetate scanning. Alternatively, the NIS mutation(s) in the index case can be determined, which then can be used for genetic testing of the kindred. Another benefit of identifying NIS mutations in ITD patients is to help identify the amino acid residues that are important for NIS structure, processing, and function (38–40).

NIS autoantibodies are found in the sera of patients with autoimmune thyroid disease (AITD), including both Graves' disease and Hashimoto's thyroiditis (41–45). However, there is difficulty understanding their role, if any, in the pathogenesis of AITD. Their incidence appears to be higher in Graves' disease than Hashimoto's thyroiditis, although NIS autoantibodies typically inhibit NIS function in cultured cells, which is not consistent with the increased radioiodine uptake of Graves' disease. If NIS autoantibodies elicit a biological response in nonthyroidal cells expressing NIS, there are no clinical symptoms of it, for instance in the salivary gland and stomach. It is unlikely, but possible, that NIS autoantibodies only recognize thyroidal NIS, due to the difference in extrathyroidal NIS glycosylation. From a clinical standpoint, it is important to determine whether serum NIS

autoantibody titers correlate with the severity and progression of AITD.

The specificity and sensitivity of tests used to measure serum NIS antibody are uncertain, and it is possible that differences in epitopes could play an important role in modulating NIS function. Because NIS may share local homologies with other proteins, it is possible that the autoantibodies detected in the sera of patients with AITD are not NIS autoantibodies. The specificity of NIS autoantibodies is especially uncertain if they do not elicit an effect on cells that express NIS. Moreover, because thyroidal NIS may be subject to alternative processing such as glycosylation and degradation, which might be different in physiological and pathological conditions, it is possible that the NIS proteins generated *in vitro* are inadequate to screen for NIS autoantibodies in patients with AITD.

NIS expression/activity can be inhibited by various cytokines, which may contribute to the development of overt hypothyroidism in patients with Hashimoto's thyroiditis. Indeed, Caturegli et al. (46) found significant inhibition of thyroidal NIS expression in transgenic mice that develop hypothyroidism after thyroid-targeted expression of interferon. Therefore, it may be of clinical interest to correlate NIS expression with the profile of cytokines, and then to correlate both with the progression of autoimmune hypothyroidism.

Thyroidal NIS forms the molecular basis for thyroid scintigraphy, using either radioiodine or ^{99m}Tc pertechnetate. ^{99m}Tc pertechnetate scans are typically performed about 20 minutes after its administration to detect uptake (NIS activity), while radioiodine scans are often performed 24 to 72 hours after its administration, thus detecting radioiodine accumulation contributed by iodide uptake (NIS activity) and iodide organification (thyroperoxidase activity). Regardless of this difference, uptake of the two isotopes is usually similar in thyroid nodules; however, a nodule may concentrate only one of the isotopes and not the other. The molecular basis for a nodule concentrating ^{99m}Tc pertechnetate but not radioiodine may be an iodide organification defect that results in the rapid release of radioiodine from the thyroid gland. However, the opposite situation in which only radioiodine is concentrated is more difficult to explain, but perhaps results from weak NIS activity with efficient iodide organification. It may be possible to compare the expression levels of NIS with those of thyroperoxidase and thyroglobulin in fine needle aspirates to uncover the molecular basis of these unusual nodules.

Thyroidal NIS may play a role in acute and chronic nonthyroid illnesses (NTI) that result in thyroid dysfunction. Enhanced cytokine secretion in chronically ill patients may influence thyroid function (35), through downregulation of NIS expression. The TSH-like activity of ethanol that induces radioiodine uptake in porcine thyrocytes warrants further study in patients with chronic alcoholism (47). Bile acid also causes lower iodide uptake in porcine thyrocyte, but it is difficult to know how this might cause hypothyroidism commonly encountered in chronic hepatic disease (48). The investigation of NIS regulation in NTI patients not only might provide insights into the cause of thyroid dysfunction, it might also help uncover new compounds and signaling pathways that regulate NIS expression and activity, which is of major importance in the use of diagnostic and therapeutic radioiodine.

NIS and Thyroid Cancers

We and others have shown that NIS expression correlates well with iodide uptake in thyroid tissue (for review see Jhiang [1]). It is thus important to investigate whether NIS expression in surgically resected tumors predicts the effectiveness of radioiodine therapy for residual and recurrent thyroid cancer. However, serum TSH levels are usually normal at the time of thyroidectomy, but greatly elevated prior to radioiodine therapy. NIS expression and activity are strongly stimulated by TSH, and it thus may not be feasible to use basal NIS expression in surgical samples to predict the intensity of TSH-stimulated NIS expression. Furthermore, the effectiveness of radioiodine therapy may not be apparent for many years. To overcome some of these problems, in addition to determining the NIS expression in surgical samples, we have established three-dimensional (3-D) histocultures of the surgical samples to evaluate their TSH-stimulated iodide uptake activity *in vitro* and found a good correlation between the two. We also found a patient with ^{18}F-flourodeoxyglucose positive and radioiodine negative lymph node metastases of papillary thyroid carcinoma that lacks basal NIS expression (Shen et al., unpublished data). Lin et al. (49) reported a case of ^{201}Tl-avid, but ^{131}I-negative metastatic thyroid cancer devoid of NIS expression (49). Therefore, the lack of NIS expression in surgical samples may be a harbinger of a poor response to radioiodine therapy, although the converse, NIS expression in surgical samples may not necessarily predict a response to radioiodine. Moreover, NIS expression levels in the primary tumor surgical specimen may not be similar to those of residual, recurrent, or metastatic cancer cells (50). Metastases may undergo dedifferentiation, and lose NIS expression/activity completely or NIS expression/activity may fail to respond sufficiently to TSH for effective radioiodine therapy.

A serum TSH level greater than 30 mIU/L is generally regarded as sufficient to stimulate radioiodine uptake for diagnostic or therapeutic purposes. However, patients do not benefit from radioiodine therapy if their TSH-stimulated NIS expression is insufficient, which may be due to a defect in NIS regulation or a defect in TSH-stimulation pathway. Theoretically, different strategies may be necessary to restore sufficient NIS activity to effectively treat thyroid cancers that do not concentrate radioiodine. Understanding the mechanisms underlying NIS regulation by TSH and other regulatory factors will help in the quest for novel reagents that might increase NIS expression in thyroid cancers that have a poor TSH-stimulated radioiodine uptake. To accomplish maximal expression of NIS in thyroid cancer, it will be necessary not only to stimulate NIS expression but also to remove NIS expression inhibitors.

In tumors that do not concentrate radioiodine, retinoic acid has occasionally induced tumor differentiation sufficiently to restore iodine uptake, perhaps by stimulating NIS expression. Retinoic acid is an important regulator of embryonic development, especially of the limbs and organs. In addition to its wide dermatological use, its potential salutary effects on tumor differentiation have been tested extensively in hematological malignancies. More recently its effects have been tested in several patients with thyroid cancer that did not or only weakly concentrated radioiodine (for review see Schmutzler and Koehrle [23]). Although few patients who

responded to retinoic acid treatment had substantially increased radioiodine uptake in their tumors, most did not. *In vitro* studies have shown that retinoids inhibit growth but increase iodide uptake in thyroid cancer cells (21,22) and increase NIS expression only in malignant thyroid cells but not in non-transformed thyroid cells (22). Stimulation of NIS expression by retinoic acid is not restricted to thyroid cancer cells, but also was shown to increase iodide uptake as much as 9.4-fold and to increase NIS expression in an estrogen receptor positive breast cancer cell line (51). Retinoic acid appears to be a promising reagent to induce radioiodine uptake in thyroid cancers and breast cancers, but clinical trials of its use in thyroid cancer to date have been disappointing.

NIS and Gastric Diseases

For some time, parietal cells were believed to actively take up radioiodine or ^{99m}Tc pertechnetate from serum and release the isotopes into the stomach (52). This was based on earlier investigations showing that the secretion of gastric acid is correlated with ^{99m}Tc pertechnetate uptake, and autoradiographic localization of ^{99m}Tc pertechnetate to the parietal cells. However, this cannot explain the gastric ^{99m}Tc pertechnetate uptake that occurs in some patients with paucity or complete lack of parietal cells as occurs with pernicious anemia. Williams and Croft (53) reported that ^{99m}Tc pertechnetate uptake occurs primarily in the gastric mucous cells, because of their preliminary finding that ^{99m}Tc pertechnetate uptake in the stomach increased in patients with gastric ulcer after treatment with carbenoxolone to normalize mucous cells. Furthermore, some newer autoradiographic studies localize ^{99m}Tc pertechnetate uptake to the mucous cells (54). Our immunohistochemical staining study showed that NIS protein is mainly detected in the basolateral membrane of the mucus secretory surface cells (1). However, others reported that NIS immunoreactivity is confined to parietal cells (55,56). This discrepancy may be caused by the differing specificity of NIS antibodies, the preparation of tissue samples, and/or the stringency of the binding conditions. It is noteworthy that gastric parietal cells, salivary acinar cells, and hepatocytes have high endogenous biotin levels that may result in nonspecific granule staining. Determining the cell type(s) that have gastric NIS expression may provide a better understanding of the regulatory mechanisms for NIS.

Although gastric radioiodine uptake is well documented, the physiological role of gastric NIS is yet to be determined. Nonetheless, adverse gastric effects have not been recognized in patients with ITD, and the regulatory mechanisms of gastric NIS are poorly understood. Because radioiodine is actively transported from the bloodstream to the gastric mucosa where it is not organified, it is rapidly released into the stomach. An earlier study showed that gastric mucosal uptake and release of ^{99m}Tc pertechnetate paralleled gastric fluid output, which is stimulated by gastrin and glucagon but suppressed by secretin (57). Whether gastric NIS expression is modulated by gastrin, glucagon, and secretin is unknown. If gastric NIS expression could be decreased, some of the side effects (nausea and vomiting) of radioiodine therapy might be minimized. Moreover, it is possible that increasing gastric NIS might facilitate the use of radioiodine for the diagnosis and therapy of gastric disease.

^{99m}Tc pertechnetate scanning has been used to diagnose several gastric diseases. Ectopic gastric mucosa, found in 30% of asymptomatic and 60% of symptomatic patients with Meckel's diverticulum, can be detected by ^{99m}Tc pertechnetate scan. In addition, scans using ^{99m}Tc pertechnetate may detect retained gastric antrum, a complication of Billroth II gastrojejunostomy, and Barrett's esophagus, a condition caused by chronic gastroesophageal reflux. To improve the sensitivity of ^{99m}Tc pertechnetate scans in detecting ectopic or inflamed gastric mucosa, pentagastrin, glucagon and cimetidine are recommended (58). It is possible that gastric NIS expression/activity is increased by pentagastrin, glucagon and cimetidine stimulation of gastric mucous cells. If gastric NIS expression can be differentially increased sufficiently, some patients with gastric cancers might benefit from ^{99m}Tc pertechnetate scanning diagnostically and/or radioiodine as therapy. Wu et al. (59) reported a case of disseminated metastatic gastric adenocarcinoma with intensive radioiodine uptake. Our follow-up study showed that some metastatic bone lesions of gastric adenocarcinoma have NIS expression by RT-PCR (Shen et al, unpublished data).

NIS and Salivary Gland Disorders

The cell type that expresses NIS in salivary glands is also the subject of controversy. The transport of ^{99m}Tc pertechnetate was demonstrated in rat parotid acinar cells both *in vitro* and *in vivo* (60). It was suggested that ^{99m}Tc pertechnetate could substitute for Cl^- as a substrate for the $Na^+/K^+/Cl^-$ cotransporter that also mediates uptake of ^{99m}Tc by rat parotid acinar cells (61). However, the unique feature of radioiodine or ^{99m}Tc pertechnetate accumulation in Warthin's tumor and oncocytoma (tumors derived from the tubular cell rather than acinar cell) indicates that radioiodine and ^{99m}Tc pertechnetate are transported by ductal epithelial cells (oncocytes) of the salivary glands, not by acinar cells (62). We and others support this notion by showing that NIS immunohistochemical staining is mainly detected in the basolateral membrane of the ductal cells, instead of in acinar cells (55,63). Taking into consideration that salivary gland radioiodine or ^{99m}Tc pertechnetate uptake is absent in patients with ITD and uptake of both isotopes is normally blocked by $ClO4^-$, it is clear that radioiodine uptake activity in salivary gland is mediated by NIS in ductal cells rather than by $Na^+/K^+/2Cl^-$ cotransporter in acinar cells.

Salivary ^{99m}Tc pertechnetate scintiscanning is a useful diagnostic aid to evaluate salivary function of patients with xerostomia (dry mouth) (64) and with some salivary tumors (65). One of the common diseases resulting in xerostomia is chronic sialadenitis caused by Sjögren's syndrome. Scans with ^{99m}Tc pertechnetate are very useful in staging Sjögren's disease: ^{99m}Tc pertechnetate uptake is intact in its early stages, decreased in its middle stage, and absent in the late stages (66), which may simply reflect the extent to which ductal cells are destroyed as the disease progresses. There is great interest in the regulatory mechanisms underlying NIS expression in salivary ductal cells. It may be possible to decrease salivary gland NIS expression to minimize the side effects of radioiodine therapy for thyroid cancer. Differentially increased salivary gland NIS expression may facilitate the use of radioiodine in the diagnosis and treatment of salivary gland diseases.

NIS and Breast Cancers

Radioiodine uptake occurs in lactating breasts, and in some cases can be more pronounced than that in a normal thyroid remnant (67). We found immunohistochemical staining for NIS was mainly on the basolateral membrane of alveolar cells and the small ductal epithelial cells in the lactating mammary gland (68). This supports the notion that iodide is transported from blood to alveolar and small ductal cells, then moved to the lumen where milk is accumulated and secreted. However, neither breast feeding nor high serum prolactin levels are required for radioiodine uptake in breasts (9,69), and bromocriptine fails to alter uptake in nonlactating breasts (9). An animal study showed that the male mammary gland may also take up ^{99m}Tc pertechnetate provided gynecomastia is induced by medroxyprogesterone acetate (70). In humans, inflammation (mastitis) and benign nonproliferative fibrocystic disease induce radioiodine uptake in breasts (69). It is also noteworthy that the nonlactating breast has a relatively large capacity for iodide uptake (or rapid turnover of iodide), as its uptake is not suppressed by iodinated contrast media (9). Although radioiodine breast uptake was found in only about 6% of nonbreast-feeding women, it can be misinterpreted as thyroid cancer lung metastases if it presents with an atypical pattern or is clinically unexpected (9). The mechanism(s) of breast radioiodine uptake in nonlactating women may be different from those of lactating women. Nevertheless, significantly increased radioiodine uptake in the lactating breast suggests that radioiodine uptake and NIS expression are subjected to hormonal control (68,71). Therefore, patients with breast cancer might benefit from radioiodine therapy if NIS expression/activity can be increased in the malignant tissues to levels sufficient for therapy.

Radioiodine uptake is increased in mouse breast tissues that show atypia or malignancy (72). One study in mice indicates that high iodide uptake may prove to be the most specific biochemical characteristic of hormone-dependent breast tumors compared to hormone-independent tumors (73). Furthermore, NIS expression and radioiodine uptake were demonstrable in two different transgenic mouse mammary tumors (71). Indeed, some human breast cancers can be detected by radionuclide scintigraphy (74,75). A recent study (71) and unpublished data from our collaborative study indicate that NIS expression is detectable by immunohistochemical staining in some human breast cancers. Taken together, these findings indicate that NIS expression is increased in lactating breast tissues and in some breast tumors as compared with normal nonlactating breast.

Analogous to thyroid cancers undergoing intensive TSH stimulation, under optimal stimulation of currently unknown hormone(s), radioiodine might be used both as a diagnostic test and for therapy of breast cancer. However, NIS expression in breast tissues appears to be driven by a combination of hormonal factors. We and others have shown that mammary gland NIS expression is regulated by prolactin and oxytocin (68,71). Retinoic acid also stimulates NIS expression and radioiodine uptake in a breast cancer cell line, MCF-7 (51). However, the increase in mammary NIS expression/activity induced by drugs and hormones is not comparable to that induced by TSH in thyroid tissues. A thorough understanding of the hormonal regulation of NIS expression/activity in breast tissues is required before an optimal regimen of its hormonal stimulation can be developed to explore the possibility of radioiodine therapy of breast cancer. At the same time, it will be important to investigate whether the selected drugs/hormones stimulate tumor growth.

NIS Gene Transfer and Cancer Therapy

We and others have shown that gene transfer of NIS into a variety of cell types confers increased radioiodine uptake up to several hundred-fold that of controls (76–80). Because NIS activity is the molecular basis of radioiodine therapy, there is great interest in exploring the possibility of NIS gene transfer to facilitate radioiodine therapy for nonthyroidal human cancers. Indeed, many investigators have demonstrated that NIS gene transfer mediated by nonviral or viral vectors into a variety of cells renders them susceptible to being killed by ^{131}I *in vitro* (78,79). Boland et al. (79) also showed that tumor expressing NIS, after intratumoral injection of recombinant adenovirus carrying NIS, may be imaged by planar scintigraphy. However, to our knowledge, a therapeutic effect of ^{131}I on animals bearing tumor expressing exogenous NIS has not been reported. The ability of radioiodine to eradicate cancer depends on the ^{131}I activity delivered to the tumors and its size. It is likely that radioiodine would be most effective in treating small tumors that may not be detectable by conventional x-ray. Our ongoing studies show that rats bearing intracranial gliomas that express exogenous NIS survive longer after ^{131}I treatment compared with untreated controls and ^{131}I treated rats bearing intracranial glioma that did not express exogenous NIS (Cho JY et al., unpublished data).

The strategy of facilitating radioiodine therapy by NIS gene transfer is particularly attractive in tumors sensitive to low-dose radiotherapy. Radioiodine therapy for thyroid cancer is generally regarded as superior to external beam radiation and is more selective in killing thyroid cancer rather than nonthyroid normal cells. Moreover, there is a choice of radioiodine isotopes that theoretically could provide different killing ranges. ^{131}I is primarily an β-emitter with a longer half-life that can deliver large amounts of radiation to tumor cells within 2 to 3 mm. ^{125}I and ^{123}I both emit auger electrons that may deposit more energy than ^{131}I within a single cell range. Thus the choice of therapeutic radioiodine isotopes might be individualized according to tumor size.

Iodide organification facilitates radioiodine retention in thyroid cancer cells, thus enhancing its therapeutic efficacy. This is unlikely to occur in nonthyroidal cancer cells that express exogenous NIS, although sufficient radiation damage occurs to salivary glands after ^{131}I therapy to cause cell destruction despite the absence of iodide organification. This suggests that the duration of radioiodine retention in a cell that is necessary to cause death is neither certain nor able to be generalized. Moreover, a series of ^{123}I gamma camera imaging studies showed that an intracranial glioma expressing exogenous NIS was detectable 37 hours after ^{123}I administration (Cho JY et al., unpublished data). Radioiodine retention in cancer cells expressing exogenous NIS but not containing the mechanism for organification thus might be sufficient for therapeutic purposes. Furthermore, pharmacological modulation of cellular efflux may offer an al-

ternative approach to increase radioiodine retention in targeted cancer cells. For example, this might be done with lithium, which increases radioiodine retention in thyroid cancer. It is also possible to introduce the thyroperoxidase gene along with NIS to introduce iodide organification into targeted cells.

Patients with thyroid cancer typically have undergone total or near-total thyroidectomy before being treated with radioiodine; however, patients who undergo NIS gene transfer in preparation for radioiodine therapy would have a functioning thyroid. Because normal thyroid cells are likely to accumulate and retain radioiodine far more efficiently than the target cancer cells that express exogenous NIS, the normal thyroid might sequester most of the radioiodine administered until it is destroyed. Using our intracranial glioma rat model, feeding the animals a T_4-supplemented diet sufficient to lower serum TSH and lower thyroidal NIS expression protected the thyroid gland during radioiodine therapy (Cho JY et al., unpublished data).

Because the NIS gene can be introduced into the tumor remaining in the surgical cavity at the time of operation, local NIS gene transfer might be very effective in facilitating radioiodine ablation of the unresected residual cancer cells. However, using radioiodine to detect and to treat distant micrometastases that do not express sufficient endogenous NIS requires improved efficiency of systemic gene transfer. Targeting NIS gene transfer and expression to the desired tumor cells and not other cells is pivotal for the success of either local gene transfer or systemic gene transfer, to eliminate unwanted side effects. Vector and transcriptional targeting can accomplish this. For example, it is possible to modify the tropism of the viral vector carrying NIS so that it can only infect the targeted cancer cells. For transcriptional targeting, a tissue specific promoter can be used to drive NIS expression only in the targeted tissues (80). Because many tumors are derived from tissues that are not necessary for life, such as thyroid, breast and prostate, it is not necessary to distinguish tumor from normal tissues. Although a tissue-specific promoter is usually not potent enough to drive NIS expression for therapeutic purpose, there are strategies to enhance tissue-specific expression of the gene of interest (81).

NIS as Imaging Reporter Gene

A vital step in transgenic animal study and gene therapy clinical trials is the ability to monitor the extent of transgene expression. Therefore, it is pertinent to develop techniques to noninvasively and repetitively determine the location, duration, and magnitude of transgene expression in living animals. Because the radionuclide approach, such as single photon emission computed tomography (SPECT) or PET, has sufficient sensitivity to quantitatively measure the gene expression *in vivo*, the approach of using imaging reporter genes with high-resolution PET scanning has been investigated (for reviews see Gambhir et al. [82] and MacLaren et al. [83]). This approach uses reporter gene coexpressed with the transgene, in which the expressions of both are closely associated. Because a radiolabeled probe can monitor the ex-

Table 3. Comparison of NIS with Other Imaging Reporter Genes

Features	*NIS*	*D_2R*	*$SSTR_2$*
Size of cDNA (kb)	~2.0	~2.5	~3.5
Immunogenicity	No	No	No
Nature of reporter	Transporter	Receptor	Receptor
Biodistribution of endogenous reporter	Thyroid, stomach, salivary gland, choroid plexus, etc.	Brain (striatum, pituitary), ganglion, adrenal gland, vessels.[94,95]	Brain, pituitary, pancreas, stomach, kidneys, other neuroendocrine tissues.[96]
Reporter toxicity	Unknown	Motor neuron disorder[97]	Unknown
Radio-labeled probes for conventional scintiscan and SPECT	^{123}I-NaI, ^{99m}Tc pertechnetate	^{123}I-IBZM	Octreotide-based analogues (e.g., ^{111}In-Octreotide, ^{99m}Tc-Depreotide)
Radiolabeled probe for PET	^{124}I-NaI	^{18}F-FESP	
Organs that accumulate radio-labeled probe	Common: thyroid, stomach, salivary gland, kidney, bladder Uncommon: breast, thymus, lacrimal gland, etc.	Common: brain, liver, lung, guts, kidney, bladder[98] Uncommon: gallbladder, melanoma[99], etc.	Common: liver, spleen, bowel, kidney, bladder[100] Uncommon: neuroendocrine and nonneuroendocrine tumors
Probe toxicity (tracer dose)	No	No	No
Radiolabeled probe preparation	Easy	Expensive	Expensive and complicated

NIS, sodium iodide symporter; D_2R, dopaminergic receptor; $SSTR_2$, type II somatostatin receptor; IBZM, iodobenzamide; FESP, fluoro-ethyl-spiperone. References are indicated by superscript numbers.

pression profile of the reporter gene, the associated transgene expression is indirectly monitored. Gambhir et al. (82) has listed the ideal characteristics of an *in vivo* imaging reporter gene and concludes that no single gene fits all the criteria (83). The dopaminergic receptor gene (D_2R) (84) and the type II somatostatin receptor ($SSTR_2$) (85,86) have been presented as possible imaging reporter genes due to their well-established imaging procedures. Other possible imaging reporter genes, such as the dopamine transporter (DT) and the glucose transporter type 2 (Glut-2), are of limited use due to their larger sizes of cDNA (larger than 7 kb).

We propose that NIS may serve as an alternative imaging reporter gene; its unique features compare favorably with those of D_2R and $SSTR_2$ (Table 3). The feasibility of imaging NIS expression has been already demonstrated using ^{123}I or ^{99m}Tc pertechnetate planar scintiscan (79; Cho JY et al., unpublished data). NIS has many advantages as an imaging reporter gene due to the wide availability of its substrate, radioiodine, and the well-understood metabolism and clearance of radioiodine in the body. Besides, there is no problem of labeling stability in using radioiodine or ^{99m}Tc pertechnetate while it may be a major concern for the radiolabeled ligands of D_2R and $SSTR_2$. Similar to the limitation of using D_2R and $SSTR_2$ as imaging reporter genes, NIS has a characteristic normal biodistribution, and therefore, may not be used to monitor transgene expression in thyroid, gastric mucosa, and the salivary gland because of their normal endogenous NIS expression. Using NIS as an image reporter gene may be further explored by investigating its sensitivity using ^{123}I or ^{99m}Tc pertechnetate (by planar camera or SPECT) or ^{124}I (by PET) in the detection and quantitation of NIS expression.

Concluding Remarks

Prior to NIS molecular characterization, clinicians have for several decades used radioiodine to diagnose and treat thyroid cancers, hyperthyroidism, and large euthyroid goiters. ^{99m}Tc pertechnetate has also been used to evaluate the secretory function of the salivary glands, and to diagnose gastric diseases caused by ectopic gastric mucosa. Further study of the molecular mechanism(s) underlying NIS regulation in various cell types may allow us to achieve maximal NIS expression/activity in target tissues yet minimize it in nontarget tissues. For thyroid cancers that no longer express sufficient NIS activity, NIS gene transfer or tumor redifferentiation might restore it. Differentially increasing NIS expression in extrathyroidal tissues selectively might permit the use of radioiodine in diagnosis and therapy of diseases and cancers derived form these tissues. However, the therapeutic efficacy of radioiodine for patients with nonthyroidal cancers expressing high levels of endogenous or exogenous NIS remains theoretical. Tumors derived from dispensable organs that retained radioiodine and are susceptible to radioiodine killing are better candidates for radioiodine ablation therapy. As a short term goal, the clinical applications of hNIS gene transfer is most promising to facilitate radioiodine ablation of locally invasive cancer cells that can not be completely resected surgically.

References

1. Jhiang SM 2000 Regulation of sodium iodide symporter. Rev Endocr Metab Disord **1**:205–215.
2. Vieja ADL, Dohan O, Levy O, Carrasco N 2000 Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. Physiol Rev **80**:1083–1105.
3. Venkataraman MG, Yatin M, Ain KB 1998 Cloning of the human sodium-iodide symporter promoter and characterization in a differentiated human thyroid cell line, KAT-50. Thyroid **8**:63–69.
4. Endo T, Kaneshige M, Nakazato M, Ohmori M, Harri N, Onaya T 1997 Thyroid transcription factor-1 activated the promoter activity of rat Na^+/I^- symporter gene. Mol Endocrinol **11**:1747–1755.
5. Tong Q, Ryu KY, Jhiang SM 1997 Promoter characterization of the rat Na^+/I^- symporter gene. Biochem Biophys Res Commun **239**:34–41.
6. Ryu KY, Tong Q, Jhiang SM 1998. Promoter characterization of the human Na^+/I^- symporter. J Clin Endocrinol Metab **83**:3247–3251.
7. Behr M, Schmitt TL, Espinoza CR, Loos U 1998. Cloning of a functional promoter of the human sodium/iodide symporter gene. Biochem J **331**:359–363.
8. Ohno M, Zannini M, Levy O, Carrasco N, Di Lauro R 1999. The paired-domain transcription factor Pax8 binds to the upstream enhancer of the art sodium/iodide symporter gene and participates in both thyroid-specific and cyclic-AMP-dependent transcription. Mol Cell Biol **19**:2051–2060.
9. Muhammad M, Hammami M, Bakheet S 1995 Radioiodine breast uptake in non-breastfeeding women: clinical and scintigraphic characteristics. J Nucl Med **37**:26–31.
10. Bakheet SM, Hammami MM, Hemidan A, Powe JE, Bajaafar F 1998 Radioiodine secretion in tears. J Nucl Med **39**:1452–1454.
11. Leder O 1982 The significance of extrathyroidal radioactive iodine accumulation and secretion in clinical pathology. Histochemistry **74**:585–588.
12. Davidson J, McDougall IR 2000 How frequently is the thymus seen on whole-body iodine-131 diagnostic and post-treatment scans? Eur J Nucl Med **27**:425–430.
13. Wilson LM, Barrington SF, Morrison ID, Kettle AG, O'Doherty MJ, Coakley AJ 1998 Therapeutic implications of thymic uptake of radioiodine in thyroid carcinoma. Eur J Nucl Med **25**:622–628.
14. Spitzweg C, Joba W, Morris JC, Heufelder AE 1999 Regulation of sodium iodide symporter gene expression in FRTL-5 rat thyroid cells. Thyroid **9**:821–830.
15. Takiyama Y, Tanaka H, Takiyama Y, Makino I 1994 The effects of hydrocortisone and RU 486 (Mifepristone) on iodide uptake in porcine thyroid cells in primary culture. Endocrinology **135**:1972–1979.
16. Furlanetto TW, Nguyen LQ, Jameson JL 1999 Estrodiol increases proliferation and down-regulates the sodium/iodide symporter gene in FRTL-5 cells. Endocrinology **140**:5705–5711.
17. Rao RH, McCready VR, Spathis GS 1986 Iodine kinetic studies during amiodarone treatment. J Clin Endocrinol Metab **62**:563–568.
18. Mettler FA, Guiberteau MJ 1991 Essentials of Nuclear Medicine Imaging, 3rd ed. W. B. Saunders, Philadelphia, p 79.
19. Vroye L, Beauwens R, Van Sande J, Dazole D, Braekman JC, Golstein PE 1998 The Na^+/I^- cotransporter of the thy-

roid: characterization of new inhibitors. Eur J Physiol **435**:259–266.
20. Harii N, Endo T, Ohmori M, Onaya T 1999 Extracellular adenosine increases Na^+/I^- symporter gene expression in rat thyroid FRTL-5 cells. Mol Cell Endocrinol **157**:31–39.
21. Van Herle AJ, Agatep ML, Padua III DN, Totanes TL, Canlapan DV, Van Herle HML, Tuillard GJF 1990 Effects of 13 *cis*-Retinoic acid on growth and differentiation of human follicular carcinoma cells (UCLA R0 82 W-1) in vitro. J Clin Endocrinol Metab **71**:755–763.
22. Schmutzler C, Winzer R, Meissner-Weigl J, Koehrle J 1997 Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. Biochem Biophys Res Commun **240**:832–838.
23. Schmutzler C, Koehrle J 2000. Retinoic acid redifferentiation therapy for thyroid cancer. Thyroid **10**:393–406.
24. Saji M, Kohn L 1991 Insulin and insulin-like growth factor-1 inhibit thyrotropin-increased iodide transporter in serum-depleted FRTL-5 rat thyroid cells: modulation of adenosine 3′,5′-monophosphate signal action. Endocrinology **128**:1136–1143.
25. Trapasso F, Iuliano R, Chiefari E, Arturi F, Stella A, Filetti S, Fusco A, Russo D 1999 Iodide symporter gene expression in normal and transformed rat thyroid cells. Eur J Endocrinology **140**:447–451.
26. Huang SS, Cerullo MA, Huang FW, Huang JS 1998 Activated thyroglobulin possesses a transforming growth factor-beta activity. J Biol Chem **273**:26036–26041.
27. Tsushima T, Arai M, Saji M, Ohba Y, Murakami H, Ohmura E, Sato K, Shizume K 1988 Effects of transforming growth factor-beta on deoxyribonucleic acid synthesis and iodine metabolism in porcine thyroid cells in culture. Endocrinology **123**:1187–1194.
28. Arai M, Tsushima T, Isozaki O, Demura H, Shizume K, Emoto N, Miyakawa M, Nozoe Y, Murakami H, Ohmura E 1995 Effects of transforming growth factor alpha (TGF-alpha) on DNA synthesis and thyrotropin-induced iodide metabolism in cultured porcine thyroid cells. Eur J Endocrinol **132**:242–248.
29. Isozaki O, Tsushima T, Miyakawa M, Emoto N, Demura H, Arai M, Sato-Nozoe Y 1997 Oncostatin M: A new potent inhibitor of iodine metabolism inhibits thyroid peroxidase gene expression but not DNA synthesis in porcine thyroid cells in culture. Thyroid **7**:71–77.
30. Kawaguchi A, Ikeda M, Endo T, Kogai T, Miyazaki A, Onaya T 1997 Transforming growth factor-beta1 suppresses thyrotropin-induced Na^+/I^- symporter messenger RNA and protein level in FRTL-5 rat thyroid cells. Thyroid **7**:789–794.
31. Pekary AE, Hershman JM 1998 Tumor necrosis factor, ceramide, transforming growth factor-beta1, and aging reduce Na^+/I^- symporter messenger ribonucleic acid levels in FRTL-5 cells. Endocrinology **139**:703–712.
32. Ajjan RA, Watson PF, Findlay C, Matcalfe RA, Crisp M, Ludgate M, Weetman AP 1998 The sodium iodide symporter gene and its regulation by cytokines found in autoimmunity. J Endocrinol **158**:351–358.
33. Sachithanandan S, Clarke G, Crowe J, Fielding JF 1997 Interferon-associated thyroid dysfunction in anti-D-related chronic hepatitis C. J Interferon Cytokine Res **17**:409–411.
34. Schmitt K, Homopesch BC, Oeland K, von Staehr WG, Thurmann PA 1999 Autoimmune thyroiditis and myelosuppression following treatment with interferon-alpha for hepatitis C. Int J Clin Pharmacol Ther **37**:165–167.
35. Yokoe T, Iino Y, Takei H, Horiguchi J, Koibuchi Y, Maemura M, Ohwada S, Morishita Y 1997 Changes of cytokines and thyroid function in patients with recurrent breast cancer. Anticancer Res **17**:695–699.
36. Pohlenz J, Refetoff S 1999 Mutations in the sodium/iodide symporter (NIS) gene as a cause for iodide transport defects and congenital hypothyroidism. Biochimie **81**:469–476.
37. Arnow JR, Oates E, Sadeghi-Nejad A 1994 Salivary gland radionucleide imaging abnormalities in infants with congenital hypothyroidism. Arch Pediatr Adolesc Med **148**:324–326
38. Levy O, De La Vieja A, Levy D, Carrasco N 1998 Identification of a structural requirement for thyroidal Na^+/I^- symporter (NIS) function from analysis of a mutation that causes human congenital hypothyroidism. FEBS Lett **429**:36–40.
39. Kosugi S, Bhayana S, Dean HJ 1999 A novel mutation in the sodium/iodide symporter gene in the largest family with iodide transport defect. J Clin Endocrinol Metab **84**:3248–3253.
40. Pohlenz J. Duprez L, Weiss RE, Vassart G, Refetoff S, Costagliola S 2000 Failure of membrane targeting causes the functional defect of two mutant sodium iodide symporters. J Clin Endocrinol Metab **85**:2366–2369.
41. Endo T, Kaneshige M, Nakazato M, Kogai T, Saito T, Onaya T 1996 Autoantibody against thyroid iodide transporter in the sera from patients with Hashimoto's thyroiditis possesses iodide transport inhibitory activity. Biochem Biophys Res Commun **228**:199–202
42. Morris JC, Bergert ER, Bryant W 1997 Binding of immunoglobulin G from patients with autoimmune thyroid disease to rat sodium-iodide symporter peptides: evidence for the iodide transporter as an autoantigen. Thyroid **7**:527–534.
43. Endo T, Kogai T, Nakazato M, Saito T, Kaneshige M, Onaya T 1996 Autoantibody against Na^+/I^- symporter in the sera of patients with autoimmune thyroid disease. Biochem Biophys Res Comm **224**:92–95.
44. Ajjan RA, Findlay C, Metcalfe RA, Watson PF, Crisp M, Ludgate M, Weetman AP 1998 The modulation of the human sodium iodide symporter activity by Graves' disease sera. J Clin Endocrinol Metab **83**:1217–1221.
45. Ajjan RA, Kemp EH, Waterman EA, Watson PF, Endo T, Onaya T, Weetman AP 2000 Detection of binding and blocking autoantibodies to the human sodium-iodide symporter in patients with autoimmune thyroid disease. J Clin Endocrinol Metab **85**:2020–2027.
46. Caturegli P, Hejaze, Suzuki K, Dohan O, Carrasco N, Kohn LD, Rose NR 2000 Hypothyroidism in transgenic mice expressing IFN-γ in the thyroid. Proc Natl Acad Sci USA **97**:1719–1724.
47. Nasu M, Sugawara M 1992 Ethanol has thyrotropin-like activity in cultured porcine thyroid follicles. Endocrinology **132**:155–160
48. Kanri R, Takiyama Y, Makino I 1996 Effects of bile acids on iodide uptake and deoxyribonucleic acid synthesis in porcine thyroid cells in primary culture. Thyroid **6**:467–474.
49. Lin JD, Chan EC, Chao TC, Chen KT, Hsueh C, Ho SY, Weng HF 2000 Expression of sodium iodide symporter in metastatic and follicular tissues. Ann Oncol **11**:625–629.
50. Arturi F, Russo D, Schlumberger M, Villard JA, Caillou B, Vigneri P, Wicker R, Chiefari E, Suarez HG, Filetti S 1998

Iodide symporter gene expression in human thyroid tumors. J Clin Endocrinol Metab **83**:2493–2496
51. Kogai T, Schultz JJ, Johnson LS, Huang M, Brent GA 2000 Retinoic acid induces sodium/iodide symporter gene expression and radioiodide uptake in MCF-7 breast cell line. Proc Natl Acad Sci USA **97**:8519–8524.
52. Williams JG 1983 Pertechnetate and the stomach—A continuous controversy. J Nucl Med **24**:633–636.
53. Williams JG, Croft DN 1980 Carbenoxolone and gastric Technetium-99m pertechnetate uptake in the patients with gastric ulceration. Scand J Gastroenterology Suppl **65**:29–33.
54. Chaudhuri TK, Polak JJ 1977 Autoradiographic studies of distribution in the stomach of ^{99m}Tc pertechnetate. Radiology **123**:223–224.
55. Spitzweg C, Joba W, Schriever K, Goellner JR, Morris JC, Heufelder AE 1999 Analysis of human sodium iodide symporter immunoreactivity in human exocrine glands. J Clin Endocrinol Metab **84**:4178–4184.
56. Kotani T, Ogata Y, Yamamoto I, Aratake Y, Kawano JI, Suganuma T, Ohtaki S 1998 Characterization of gastric Na^+/I^- symporter of the rat. Clin Immunol Immunopathol **89**:271–278.
57. Khettery J, Effmann E, Grand RJ, Treves S 1976 Effects of pentagastrin, histolog, glucagon, secretin, and perchlorate on the gastric handling of ^{99m}Tc pertechnetate in mice. Radiology **120**:629–631.
58. Zeissman HA 1996 The gastrointestinal tract. In: Harbert JC, Eckelman WC, Neumann RD (eds) Nuclear Medicine: Diagnosis and Therapy. Thieme Press, New York, pp 627–633.
59. Wu SY, Kollin J, Coodley E, Lockyer T, Lyons KP, Moran E, Parker LN, Yu AC 1984 I-131 total-body scan: localization of disseminated gastric adenocarcinoma. Case report and survey of the literature. J Nucl Med **25**:1204–1209.
60. Fox PC, Bonder L, Bowers MR, Baum BJ 1986 Uptake and secretion of technetium pertechnetate by the rat parotid gland. Comp Biochem Physiol **83A**:579–584.
61. Helman J, Turner RJ, Fox PC, Baum BJ 1987 ^{99m}Tc-pertechnetate uptake in parotid acinar cells by the $Na^+/K^+/Cl^-$ co-transport system. J Clin Invest **79**:1310–1313.
62. Greyson ND 1998 Salivary glands. In: Maisey MN, Britton KE, Collier BD (eds) Clinical Nuclear Medicine, 3rd ed. Chapman & Hall Medical Press, London, pp 557–565.
63. Jhiang SM, Cho JY, Ryu KY, DeYoung BR, Smanik PA, McGaughy VR, Fischer AH, Mazzaferri EL 1998 An immunohistochemical study of Na^+/I^- symporter in human thyroid tissues and salivary gland tissues. Endocrinology **139**:4416–4419.
64. Chisin R, Markitziu A, Hoffer S, Shani J, Altan H 1988 The clinical value of quantitative dynamic scintigraphy in salivary gland disorders. Int J Radiat Appl Instrum (B) **15**:313–317
65. De Klerk JM, Van Den Biezenbos AR, Cardinaal RM, Van Rijk PP 1997 Value of technetium-99m pertechnetate imaging in the differential diagnosis of salivary lesions. Ann Otol Rhinol Laryngol **106**:432–434.
66. Baum BJ, Fox PC, Neumann RD 1996 The salivary glands. In: Harbert JC, Eckelman WC, Neumann RD (eds) Nuclear Medicine: Diagnosis and Therapy. Thieme Press, New York, p 443.
67. Bakheet SM, Hammami MM 1994 Patterns of radiouptake by the lactating breast. Eur J Nucl Med **21**:604–608.
68. Cho JY, Leveille R, Kao R, Rousset B, Parlow AF, Burak WE, Mazzaferri EL, Jhiang SM 2000 Hormonal regulation of radioiodide uptake activity and Na^+/I^- symporter expression in mammary glands. J Clin Endocrinol Metab **85**:2936–2943.
69. Allen T, Wiest P, Vela S, Hartshorne M, Crooks LA 1998 I-131 uptake in the breast for thyroid cancer surveillance with biopsy-proven benign tissue. Clin Nucl Med **9**:585–587.
70. Patricelli AJ, Lappin MR, Steyn PF 1999 Mammary gland uptake of sodium Tc-pertechnetate in a cat with a drug-induced gynecomastia. Vet Radiol Ultrasound **40**:87–88.
71. Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, Deng HF, Amenta PS, Fineberg S, Pestell RG, Carrasco N 2000 The mammary gland iodide transporter is expressed during lactation and in breast cancer. Nat Med **6**:871–878.
72. Eskin BA 1977 Iodine and mammary cancer. Adv Exp Med Biol **91**:293–304.
73. Briand P 1983 Hormonal-dependent mammary tumors in mice and rats as a model for human breast cancer. Anticancer Res **3**:273–281.
74. Eskin BA, Parker FJ, Bassett JG, George DL 1974 Human breast uptake of radioactive iodine. Obstet Gynecol **44**:398–402.
75. Cancroft ET, Goldsmith SJ 1973 ^{99m}Tc pertechnetate scintigraphy as an aid in the diagnosis of breast mass. Radiology **106**:441–444.
76. Cho JY, Xing S, Liu X, Buckwalter TLF, Hwa L, Sferra TJ, Chu IM, Jhiang SM 2000 Expression and activity of human Na^+/I^- symporter in human glioma cells by adenovirus-mediated gene delivery. Gene Ther **7**:740–749.
77. Shimura H, Harrguchi K, Miyazaki A, Endo T, Onaya T 1997 Iodide uptake and experimental ^{131}I therapy in transplanted undifferentiated thyroid cancer cells expressing Na^+/I^- symporter gene. Endocrinology **138**: 4493–4496.
78. Mandell RB, Mandell LZ, Link CJ 1999 Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. Cancer Res **59**:661–668.
79. Boland A, Richard M, Opolon P, Bidart JM, Yeh P, Filetti S, Schlumberger M, Perricaudet M 2000 Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for targeted radiotherapy. Cancer Res **60**:3484–3492.
80. Spitzweg C, Zhang S, Bergert ER, Castro MR, Mclver B, Heufelder AE, Tindall DJ, Young CYF, Morris JC 1999 Prostate-specific antigen (PSA) promoter-driven androgen-inducible expression of sodium iodide symporter in prostate cancer cell lines. Cancer Res **59**:2136–2141.
81. Nagayama Y, Nishihara E, Iitaka M, Namba H, Yamashita S, Niwa M 1999 Enhanced efficacy of transcriptionally targeted suicide gene/prodrug therapy for thyroid carcinoma with the Cre-loxP system. Cancer Res **59**:3049–3052.
82. Gambhir SS, Barrio JR, Herschman HR, Phelps ME 1999 Assays for non-invasive imaging of reporter gene expression. Nucl Med Biol **26**:481–490.
83. MacLaren DC, Toyokuni T, Cherry SR, Barrio JR, Phelps ME, Herschman HR, Gambhir 2000 PET imaging of transgene expression. Biol Psychiatry **48**:337–348.
84. MacLaren DC, Gambhir SS, Satyamurthy N, Barrio JR, Sharfsteion S, Toyokuni T, Wu L, Berk AJ, Cherry SR, Phelps ME, Herschman HR 1999 Repetitive, non-invasive imaging of the dopamine D_2 receptor gene in living animals. Gene Ther **6**:785–791.
85. Gambhir SS, Barrio JR, Phelps ME, Iyer M, Namavari M, Satyamurthy N, Wu L, Green LA, Bauer E, MacLaren DC, Nguyen K, Berk AJ, Cherry SR, Herschman HR 1999 Imag-

ing adenoviral-directed reporter gene expression in living animals with positron emission tomography. Proc Natl Acad Sci USA **96**:2333–2338.

86. Zinn KR, Buchsbaum DJ, Chaudhuri TR, Mountz JM, Grizzle WE, Rogers BE 2000 Noninvasive monitoring of gene transfer using a reporter receptor imaged with a high-affinity peptide radio-labelled with ^{99m}Tc or ^{188}Re. J Nucl Med **41**:887–895.
87. Kogai T, Endo T, Saito T, Miyazaki A, Kawaguchi A, Onaya T 1997 Regulation by thyroid-stimulating hormone of sodium/iodide symporter gene expression and protein levels in FRTL-5 cells. Endocrinology **138**:2227–2232.
88. Kogai T, Crucio F, Hyman S, Cornford EM, Brent G, Hershman JM 2000 Induction of follicle formation in long-term cultured normal human thyroid cells treated with thyrotropin stimulates iodide uptake but not sodium/iodide symporter messenger RNA and protein expression. J Endocrinol **167**:125–135.
89. Saito T, Endo T, Kawaguchi A, Ikeda M, Nakazato M, Kogai T, Onaya T 1997 Increased expression of the Na^+/I^- symporter in cultured human thyroid cells exposed to thyrotropin and in Graves' thyroid tissue. J Clin Endocrinol Metab **82**:3331–3336
90. Uyttersprot N, Pelgrims N, Carrosco N, Gervy C, Maenhaut C, Dumont JE 1997 Moderate doses of iodide in vivo inhibit cell proliferation and the expression of thyroperoxidase and Na^+/I^- symporter mRNAs in dog thyroid. Mol Cell Endocrinol **131**:159–203.
91. Eng PH, Cardona GR, Fang SL, Previti M, Alex S, Carrasco N, Chin WW, Braverman LE 1999 Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein. Endocrinology **140**:3404–3010.
92. Tsuchiya Y, Saji M, Isozaki O, Arai M, Tsushima T, Shizume K 1990 Effects of lithium on deoxyribonucleic acid synthesis and iodide uptake in porcine thyroid cells in culture. Endocrinology **126**:460–465.
93. Urabe M, Hershman JM, Pang XP, Murakami S, Sugawara M 1991 Effects of lithium on function and growth of thyroid cells in vitro. Endocrinology **129**:807–814.
94. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG 1998 Dopamine receptors: from structure to function. Physiol Rev **78**:189–225.
95. Peterfreund RA, Kosofsky BE, Fink JS 1996 Cellular localization of dopamine D_2 receptor messenger RNA in the rat trigeminal ganglion. Anesth Analg **81**:1181–1185.
96. Patel YC 1999 Somatostatin and its receptor family. Front Neuroendocrinol **20**:157–198.
97. Umegaki H, Chernak JM, Ikari H, Roth GS, Ingram DK 1997 Rotational behavior produced by adenovirus-mediated gene transfer of dopamine D_2 receptor into striatum. NeuroReport **8**:3553–3558.
98. Verhoeff NPLG, Sokole EB, Stabin M, Hengst D, Kung HF, Van Royen EA, Janssen AGM 1993 Dosimetry of iodine-123 iodobenzamide in healthy volunteers. Eur J Nucl Med **20**:747–752.
99. Maffioli L, Mascheroni L, Mongioj V, Gasparini M, Baldini MT, Seregni E, Castellani MR, Cascinelli N, Buraggi GL 1994 Scintigraphic detection of melanoma metastases with a radiolabelled benzamide ([Iodine-123]-(s)-IBZM). J Nucl Med **35**:1741–1747.
100. Kwekkeboom DJ, Krenning EP 1999 Somatostatin receptor imaging in oncology. In: Tauxe WN, Aktolun C (eds) Nuclear Oncology. Springer-Verlag, New York, pp 345–348.

Address reprint requests to:
Sissy M. Jhiang
304 Hamilton Hall, 1645 Neil Avenue
Columbus, OH 43210

E-mail: jhiang.1@osu.edu