

# Immunohistochemical Analysis of Sodium Iodide Symporter Expression in Metastatic Differentiated Thyroid Cancer: Correlation with Radioiodine Uptake

M. REGINA CASTRO, ELIZABETH R. BERGERT, JOHN R. GOELLNER, IAN D. HAY, AND JOHN C. MORRIS

*Divisions of Endocrinology and Metabolism (M.R.C., E.R.B., I.D.H., J.C.M.) and Surgical Pathology and Laboratory Medicine (J.R.G.), Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905*

The ability of thyroid cancers to concentrate radioiodine (RAI) is dependent, in part, upon the expression and functional integrity of the sodium iodide symporter (NIS). However, some differentiated thyroid carcinomas (DTCs) and most undifferentiated thyroid carcinomas lack the ability to concentrate iodide and are thereby insensitive to  $^{131}\text{I}$  therapy. Variation of NIS protein expression may be an important factor in this behavior. We wished to determine whether NIS protein expression in primary DTC tumors correlated with the subsequent RAI uptake by metastatic lesions in the same patients. We obtained paraffin-embedded tissue specimens from 60 patients with metastatic thyroid cancer who had undergone total or near-total thyroidectomy at the Mayo Clinic for DTC and had known presence or absence of RAI uptake in their tumor deposits determined by total body scanning after thyroid hormone withdrawal. Tissue sections from the primary intrathyroidal tumors were subjected to immunostaining (IS) using a monoclonal antibody against human NIS. Slides were subsequently examined for specific IS by two independent reviewers. For each patient, whole body scan (WBS) uptake was recorded, and correlation between results of IS and WBS was analyzed.

Of 43 patients with a positive WBS, 37 also had positive IS of their tumors. In six patients with negative IS, a positive WBS was documented, and in three of these cases TSH at the time of surgery was less than 0.3 mIU/liter. Of the 17 patients with negative WBS, 10 were also negative on IS. Positive IS accurately predicted a positive scan in our study in 84% of cases; the ability of the IS to detect all cases with a positive scan was 86%, and it increased to 90% when patients who were receiving thyroid hormone therapy at the time of surgery were excluded from the analysis.

Overall, the results of our retrospective study suggest that NIS IS of the thyroïdal primary tumor in patients with papillary and follicular thyroid cancers has substantial ability to predict the behavior of subsequent deposits of metastatic and recurrent cancer with respect to iodine trapping and concentration. Our findings require confirmation in prospective studies to more accurately determine the predictive ability of the test and its role in the postoperative management of patients with DTC. If confirmed, NIS IS of DTC primary lesions may prove useful in the management of patients with known or suspected metastatic thyroid cancer. (*J Clin Endocrinol Metab* 86: 5627-5632, 2001)

THE ACTIVE TRANSPORT of iodide across the basolateral membrane into thyroid follicles has been the object of study for decades (1, 2). More recently, the cloning of the rat iodide transporter gene has been a major step forward (3) in the understanding of the cellular and molecular biology of the process. The gene for the human sodium iodide symporter (hNIS) has also been cloned, and its expression has been characterized in thyroid, as well as in several nonthyroidal tissues (4, 5). The ability of many well differentiated thyroid cancers to concentrate iodide allows patients with residual or metastatic disease to receive radioiodine (RAI) ablation of their tumors (6, 7). However, some differentiated cancers, as well as most undifferentiated thyroid carcinomas, lack the ability to concentrate iodide and, hence, are insensitive to  $^{131}\text{I}$  therapy (7).

The ability of these tumors to concentrate RAI is very likely dependent, at least in part, upon the expression and functional integrity of the iodide symporter. In support of this hypothesis, several groups have demonstrated variable NIS

mRNA and protein expression in various thyroid disorders, including thyroid adenomas and carcinomas, Hashimoto's thyroiditis, and Graves' disease, using methods such as RT-PCR (8, 9) and by immunocytochemical analysis, using polyclonal (10-12) and monoclonal antibodies against NIS protein (13, 14). In most reports to date, hypo- or nonfunctional thyroid tumors, whether benign or malignant, usually demonstrate reduced, although variable, levels of NIS mRNA and protein expression when compared with normal thyroid tissue, whereas tissue obtained from Graves' patients demonstrates increased expression of NIS (13, 15). Others have also found correlation between immunocytochemical expression of NIS in thyroid tumors and their ability to concentrate iodine when imaged by  $^{131}\text{I}$  uptake, although the number of patients in these studies has been small (16, 17). Furthermore, although the role of NIS in the ability of tumors to take up iodine cannot be questioned, its role in the lack of such ability has not been completely examined.

An important clinical question regarding NIS expression in thyroid cancers is whether measuring its presence or absence in a given tumor yields useful information to the clinician who is caring for that patient. In the present study, we have analyzed the immunohistochemical expression of hNIS

Abbreviations: CC, Correlation coefficient; DTC, differentiated thyroid carcinoma; FTC, follicular thyroid carcinoma; HC, Hürthle cell; hNIS, human sodium iodide symporter; IS, immunostaining; PTC, papillary thyroid carcinoma; WBS, whole body scan.

protein in a series of differentiated thyroid carcinomas (DTC) that have been previously characterized as to their iodine-trapping ability. Most importantly, we wished to determine whether examination of the primary tumor for NIS expression can predict the biologic behavior of the metastatic deposits with respect to iodine trapping, as monitored by  $^{131}\text{I}$  total body scanning. We hypothesize that variable NIS expression is responsible for much of the variability of iodine uptake activity seen in the tumors of patients with DTC.

## Materials and Methods

### Subjects

We identified and carefully reviewed the medical records of approximately 200 patients who were diagnosed with DTC (papillary or follicular cell type) and underwent total or near-total thyroidectomy, neck dissection, or excision of distant metastasis from this tumor at the Mayo Clinic (Rochester, MN). These 200 were chosen serially from a computer-generated list of patients who were identified by the diagnosis of metastatic thyroid cancer and who had received their primary surgery at Mayo Clinic. Sixty of those patients who met the following inclusion criteria were selected for the study: 1) tissue (paraffin-embedded) available from thyroid carcinoma (primary tumor) obtained from neck surgery performed at Mayo Clinic between 1966 and 1996; 2) no prior RAI therapy; 3) known metastatic disease, to regional neck lymph nodes or to distant sites (lungs, bone, *etc.*), demonstrated by surgical biopsy and/or imaging modalities other than RAI scanning (computed tomography), technetium bone scan, magnetic resonance imaging, or ultrasound) that was confirmed as present subsequent to the initial thyroid surgery (metastatic disease with negative RAI scanning was confirmed by biopsy in all cases); and 4) whole body scan (WBS) with  $^{131}\text{I}$  after thyroid hormone withdrawal performed within 3 months of the time of surgery, demonstrating the status of the tumor regarding iodine trapping.

Thus, all patients had thyroid cancer present after surgery that was documented by methods other than RAI scanning.

The following data were collected from the medical records of each patient: 1) medical record number; 2) date of surgery; 3) treatment status regarding thyroid hormone replacement or suppression at the time of the surgery from which tissue for immunostaining (IS) was collected; 4) TSH level at the time of surgery ( $T_4$  or free  $T_4$  when TSH unavailable) and at the time of  $^{131}\text{I}$  WBS; 5) sites and extent of metastases delineated by imaging procedures; 6) results (sites and number of foci, percentage of RAI tracer uptake) of  $^{131}\text{I}$  WBS as determined by nuclear medicine specialist; and 7) thyroglobulin levels at the time of surgery. Review of medical records and use of paraffin-embedded tissue blocks of these subjects was approved by the Institutional Review Board of the Mayo Foundation.

### Whole body scanning

All patients had whole body  $\gamma$  imaging performed 48 h after administration of 3 mCi  $^{131}\text{I}$  and withdrawal of  $T_4$  therapy for 6 wk. Although the instrumentation for the images was updated during the period, each instrument was considered of the best quality available at the time of its use. All patients had anterior and posterior whole body view as well as anterior and spot views of the head, neck, and chest. TSH levels above 30 mIU/liter before scanning were confirmed in all cases in which TSH assays were available.

### Immunohistochemistry

Paraffin tissue blocks from the primary tumor at initial presentation, before any  $^{131}\text{I}$  therapy, were obtained from the archives of our Pathology Department. Slides from these blocks were made, and one slide from each patient was stained with hematoxylin and eosin and reviewed by thyroid pathologist (J.R.G.) for confirmation of the original diagnosis and presence of malignancy.

The slides were deparaffinized and stained by manual immunoperoxidase method according to a previously described protocol using a monoclonal antibody against hNIS (FP-13), which recognizes the car-

boxy-terminal portion of the molecule, and which has been previously characterized by Western blot and immunohistochemistry (14). For positive control, tissue from a Graves' disease patient was used. Negative controls were performed for each sample and positive controls within each assay. Slides that showed no IS were considered negative. All others were considered positive, regardless of the percentage of cells stained or the intensity of IS.

The slides were examined for specific IS and scored independently by two of the authors (J.R.G., M.R.C.), who were blinded as to the RAI scan results and all other patient characteristics. A preliminary analysis of 60 samples by the two reviewers yielded a correlation coefficient of 0.70.

After all slides had been reviewed and scored independently and blindly, they were reviewed jointly, and differences were resolved by consensus. Results were entered into a database and compared with those of  $^{131}\text{I}$  uptake. Correlations were analyzed by Spearman rank order analysis.

## Results

### Patient and tumor characteristics

Table 1 summarizes patients' characteristics, as well as results of laboratories, WBS, and hNIS IS. Of the 60 patients selected, 33 tumors were classified as follicular thyroid carcinoma (FTC), 8 of which were Hürthle cell (HC) variant. The remaining tumors ( $n = 27$ ) were papillary thyroid carcinomas (PTCs). Sites of metastases included lungs ( $n = 29$ ), bones ( $n = 29$ ), lateral neck lymph nodes ( $n = 17$ ), adrenal glands ( $n = 2$ ), kidney ( $n = 1$ ), and liver ( $n = 1$ ). Six patients had recurrence within or near the thyroid bed. Forty percent of patients had metastases to more than one organ (most commonly lungs and bones). Forty-three patients (72%) had a positive WBS uptake; the rest had negative WBS despite known metastases outside of the thyroid bed.

Serum TSH levels obtained at Mayo Clinic at the time of surgery were available in 35 patients, and 58 patients had  $T_4$  levels documented (one patient had free  $T_4$ ). TSH levels in these 35 patients ranged from less than 0.05 to 39.3 mIU/liter. TSH levels were within the normal range (0.3 to 5.0 mIU/liter) in 27 patients, suppressed ( $<0.3$  mIU/liter) in 5 patients, and above normal ( $>5.0$  mIU/liter) in 3 (range, 5.3 to 39.3 mIU/liter). Serum  $T_4$  levels ranged from 4.1 to 16.4  $\mu\text{g/dl}$  (normal range, 5.0–12.5  $\mu\text{g/dl}$ ). Only two patients had  $T_4$  levels outside the normal range. Another patient had only free  $T_4$  levels measured at the time of surgery, which were within the normal range (normal, 0.8–1.8 ng/dl).

Fourteen patients were on thyroid hormone treatment (Cytomel, Synthroid, Armour Thyroid) at the time of surgery, yet only two of them had suppressed TSH levels. Two patients had elevated TSH levels (25.9 and 39.3 mIU/liter) and one patient had a low  $T_4$  (4.1  $\mu\text{g/dl}$ ), despite this treatment.

### Immunostaining and correlation with WBS

Of 43 patients who had a positive WBS, 37 also had positive IS of their tumors (Table 2). The percentage of cells stained as well as the intensity of the IS was highly variable and often more diffuse and poorly localized as demonstrated in previous studies, in contrast to the intense and well localized pattern seen in glands affected by Graves' disease (14). Tumors from six patients had negative IS (Fig. 1A), despite a positive WBS. Of these, two patients were receiving treatment with thyroid hormone and had a low TSH ( $\leq 0.4$  mIU/liter) documented at the time of surgery, which may

**TABLE 1.** Patient characteristics

Patient no.	Diagnosis	Scan	IS	TSH at surgery (mIU/liter)	T <sub>4</sub> (μg/dl)	TSH at scan (mIU/liter)	TG (ng/ml)	Sites of metastases
1	FTC	+	+	—	6.8	44.5	1,470	Lungs
2	FTC	—	—	—	6.6	—	2,190	Lungs, bones
3	PTC	+	+	0.91	—	14	29.4	Lungs
4 <sup>a</sup>	FTC	—	—	—	0.9 <sup>b</sup>	—	—	Bone
5	PTC	+	+	—	16.4	49.5	215	Cervical LN
6 <sup>a</sup>	FTC	+	+	1.7	—	14.6	—	Lungs
7	FTC	+	+	—	6.6	—	—	Bones, lungs, mediastinum
8	PTC	+	+	—	7.6	51	3,730	Upper mediastinum
9	FTC	+	+	—	6.3	66	—	Lungs
10 <sup>a</sup>	FTC	+	+	—	5.0	35.2	—	Bone (skull), recurrence in neck
11	FTC	+	—	—	9.4	—	—	Lungs, bones, recurrence in neck
12	PTC	+	—	1.8	—	93.7	773	Mediastinal, lungs
13	FTC	—	+	—	7.9	—	54.6	Bone (spine)
14	PTC	+	—	—	8.8	218	—	Cervical LN, Lungs
15	FTC	+	+	4.4	—	4.4	—	Bone
16 <sup>a</sup>	PTC	+	—	0.39	—	37.5	13.4	Bone (spine, humerus), neck recurrence
17 <sup>a</sup>	FTC	+	—	—	4.1	—	—	Bones (pelvis)
18	FTC	+	+	4	—	215	—	Bones (S1)
19	HC	+	+	—	7.7	136	905	Lungs, bone (scapula)
20	PTC	+	+	3.8	—	86.2	—	Lateral neck LN
21	FTC	+	+	—	12.2	—	—	Bone (spine and ribs)
22 <sup>a</sup>	PTC	+	+	1	—	186	10.4	Large recurrent tumor in neck, cervical LN
23	HC	+	+	0.4	—	67.5	1.6	Lungs, bones, cervical LN
24	PTC	+	+	—	9.4	154	—	Cervical LN, lungs
25	PTC	—	+	—	7.9	66.6	50.4	Cervical LN
26	FTC	—	—	3.3	—	5.3	3.8	Lungs, recurrence in neck
27	FTC	+	+	—	6.4	117	21.2	Lungs, bone
28	FTC	—	—	—	7.8	119	>1,000	Bones
29	PTC	—	—	—	8.5	163	—	Lungs, cervical LN
30	HC	—	+	—	8.1	Given rTSH	—	Bones (spine)
31	FTC	—	—	<1.0	—	106	—	Lungs, bones, recurrent neck tumor
32	PTC	—	+	0.3	—	118	75.6	Lungs, bones, paratracheal mass
33	PTC	+	+	1.6	—	11.3	—	Bones (hip and ribs)
34	HC	+	+	—	7.0	82.2	—	Bones (shoulder and ribs)
35	FTC	+	+	1.5	—	57.2	>1,000	Left adrenal, bone (spine)
36	FTC	+	—	<0.3	—	37.2	>1,000	Lungs, bones
37	PTC	+	+	3.1	—	103	—	Cervical LN
38	PTC	+	+	2.4	—	147	11.9	Cervical LN, bone
39 <sup>a</sup>	PTC	—	+	—	9.3	122	11.5	Lung, cervical LN
40 <sup>a</sup>	HC	—	+	25.9	—	73.1	—	Cervical LN
41	PTC	—	—	—	10.1	46.3	59.4	Cervical (jugular) LN
42	HC	—	+	3.8	—	—	—	Bilaterally around internal jugular veins
43 <sup>a</sup>	PTC	—	—	<0.05	—	15.5	281	Cervical LN
44	FTC	+	+	2	—	81.6	14.9	Bone (scapula)
45	HC	—	—	0.74	—	6.1	24,300	Lungs, mediastinum
46	PTC	+	+	—	—	117	1,115	Cervical LN
47	PTC	+	+	2.2	—	51.1	99.4	Lungs
48	FTC	+	—	0.3	—	30.6	14,500	Mediastinum, lungs
49	FTC	+	+	0.65	—	139	1,370	Cervical LN, lungs, bones
50	PTC	+	+	0.25	—	129	965	Cervical, SC-LN, lungs
51	FTC	+	+	1	—	2.8	3,720	Lungs, cervical LN, bone (hip)
52	PTC	+	+	1.3	—	54.5	17,967	Lungs, bones, left adrenal
53 <sup>a</sup>	HC	+	+	5.3	—	98.6	760	Bone (spine)
54 <sup>a</sup>	FTC	—	—	3.3	—	177	144	Kidney, liver
55	PTC	+	+	0.78	—	28.4	201	Paratracheal LN, periesophageal
56 <sup>a</sup>	PTC	+	—	0.96	—	44.3	216	Cervical LN
57 <sup>a</sup>	PTC	+	—	0.25	—	49.6	177	Bone (ribs), mediastinum, paratracheal LN
58	FTC	+	+	3.8	—	92.7	72.5	Lungs, mediastinum
59	PTC	+	+	—	—	—	—	Mediastinum
60 <sup>a</sup>	PTC	+	+	39.3	—	74.1	48	Lungs

LH, Lymph nodes; TG, serum thyroglobulin. —, Not available except for IS and scan, where it indicates negative results. rTSH, recombinant TSH; SC-LN, supraclavicular lymph nodes.

<sup>a</sup> Patients were receiving thyroid hormone at the time of surgery.

<sup>b</sup> Free T<sub>4</sub>.

**TABLE 2.** Results of IS and WBS in all patients with DTC (PTC and FTC)

All (PTC + FTC)		WBS		Total
		+	–	
IS	+	37	7	44
	–	6 <sup>a,c</sup>	10 <sup>b</sup>	16
Total		43	17	60

<sup>a</sup> Two patients and <sup>b</sup> one patient on thyroid hormone at time of surgery and suppressed TSH (<0.3 mIU/liter).

<sup>c</sup> One patient with suppressed TSH (<0.3) off thyroid hormone.

have suppressed NIS expression (18). Another patient in this group presented with hyperthyroidism (suppressed TSH <0.3 mIU/liter and elevated T<sub>4</sub> at 14 µg/dl) and widely metastatic FTC for which she underwent thyroidectomy. Of the 17 patients with negative WBS, 10 also had a negative IS.

Positive IS accurately predicted a positive scan in our study in 37 of 44 (84%) cases. However, in only 10 of 17 (59%) cases with negative WBS, the IS results were concordant. In 10 of 16 (62.5%) cases, a negative IS accurately predicted a negative WBS (Table 2). When results of IS taken either as positive or negative were correlated with results of WBS, the Spearman rank correlation coefficient (CC) for the whole group was 0.367 ( $P = 0.004$ ). The correlation between these two tests remained significant even when controlling for treatment with T<sub>4</sub> (CC = 0.360;  $P = 0.005$ ) and for type of cancer (CC = 0.365;  $P = 0.004$ ).

NIS expression in thyroid cells is dependent upon stimulation of the thyroid gland by TSH, and low TSH levels reduce NIS expression *in vitro* and *in vivo* (15, 18). Thus, thyroid hormone therapy, by way of its effect on circulating TSH levels, is likely to influence NIS expression in patients with thyroid cancer. We examined the potential influence of T<sub>4</sub> therapy on NIS expression in our study. After exclusion from the analysis of all patients who were receiving thyroid hormone therapy at the time of surgery, 84.7% of cases showed concordant results between IS and WBS (Table 3).

Of the 27 patients with PTC, 21 had positive RAI uptake at known sites of metastases, whereas 6 had negative WBS despite known metastatic disease (Table 4). Nineteen of the patients with PTC had a positive IS. As shown in Table 4, positive results of IS were observed in 16 of the 21 (76%) scan-positive tumors. After exclusion of the patients who received thyroid hormone therapy before surgery, the concordance between positive WBS and positive IS increased to 88% (14 of 16).

Of the five patients with positive WBS and negative IS, three were receiving thyroid hormone treatment at the time of surgery, and their serum TSH levels were less than 0.4 mIU/ml in two cases at the time of thyroidectomy. Overall, concordance between the WBS uptake and IS was seen in 70% of patients with PTC, and it increased to 80% by excluding patients who were receiving thyroid hormone at the time of surgery.

Of the 33 patients with FTC, 22 had a positive WBS, whereas 11 had negative WBS despite known metastatic disease. Twenty-two patients in this group had positive IS (Fig. 1, C and D), and in 18 (82%) of them this coincided with a positive WBS. Seven patients with FTC were on thyroid hormone therapy at the time of surgery, and of these, four

had positive IS. Overall, concordance between WBS uptake and IS was seen in 25 of 33 (76%) patients with FTC. The ability of a positive IS to predict a positive WBS uptake in patients with FTC was 82%. Of interest, seven of the eight HC variant FTC tumors demonstrated positive IS for NIS, but only four were associated with positive RAI scans in the metastatic deposits.

## Discussion

The ability of thyroid follicular cells to concentrate iodide is dependent upon the functional integrity of the NIS (2, 8). DTC retains this iodide-concentrating ability, which is used both diagnostically (<sup>131</sup>I scans) and therapeutically for RAI therapy of residual or metastatic thyroid cancer after initial surgical excision (6, 7). However, some differentiated thyroid tumors are incapable of concentrating iodide, which renders them nonsensitive to RAI therapy and increases the morbidity and mortality for these patients (7, 19). Furthermore, most undifferentiated thyroid carcinomas are also unable to concentrate RAI. When these tumors become metastatic, effective treatment is not currently available, which adds to the morbidity and mortality associated with this disease.

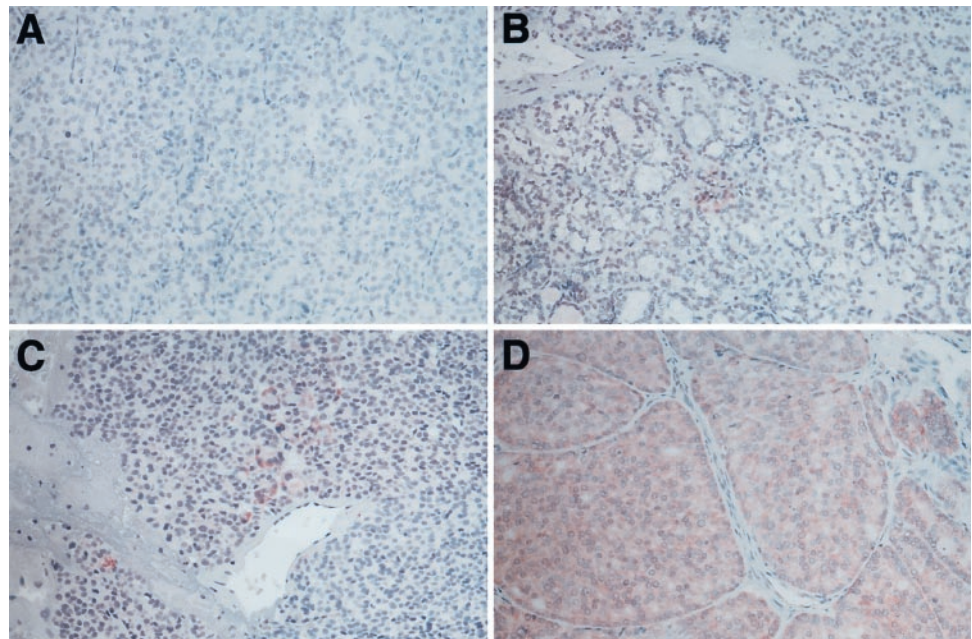
The purpose of the current study was to examine the potential clinical utility of performing immunohistochemical analysis of NIS protein expression in the primary tumor from patients with DTC. We wished to examine the ability of this analysis to predict the subsequent behavior of metastatic or recurrent deposits of that tumor with respect to trapping of RAI. To that end, we have analyzed a series of DTCs, obtained from the Mayo Clinic archives, for the ability of metastatic or recurrent disease derived from those tumors to concentrate iodide, as reflected by RAI uptake, and correlated the results with hNIS protein expression within the primary tumor by immunohistochemical analysis. The immunoassay used a specific monoclonal antibody against hNIS that was developed and characterized in our laboratory (14).

Overall, 47 of 60 (78%) tumors showed concordance between the results of <sup>131</sup>I scans in known metastatic disease and immunohistochemical hNIS expression. Positive IS accurately correlated with a positive scan in 37 of 43 (86%) cases and accurately predicted a negative WBS in 10 of 17 (59%). Thus, in 13 cases, the IS results did not correlate with the RAI scan.

Thyroid hormone therapy, when used in doses sufficient to suppress TSH, as in the treatment of thyroid cancer, may result in decreased expression of hNIS protein (15, 18). This was also observed in our study when comparing the ability of hNIS IS to predict a positive WBS in the group of patients with DTC as a whole (86%) to those not receiving thyroid hormone therapy (94%).

Several tumors ( $n = 7$ ) that were found to be positive on IS had a negative WBS uptake. This was especially true for the eight HC variant follicular cancers, which are notorious for their reduced iodine trapping ability. Potential explanations for these discordant results include: 1) abnormal glycosylation or other posttranscriptional or posttranslational modifications of the NIS protein in tumor cells, which could render the molecule nonfunctional, yet still immunoreactive;

FIG. 1. NIS IS of primary thyroid cancers. Each slide was reviewed blindly by two independent reviewers. Discrepancies were resolved by blinded consensus. A, Follicular carcinoma, negative hNIS IS. B, papillary carcinoma, weakly positive ( $\leq 25\%$  of cells stain positive for hNIS). C, follicular carcinoma, moderately positive IS (26–50% of cells stain positive for hNIS). D, follicular carcinoma, strongly positive ( $>75\%$  of cells with + IS). Magnification, A–D,  $\times 200$ .



**TABLE 3.** Results of IS and WBS in all patients with DTC (PTC and FTC), excluding those on thyroid hormone therapy at the time of surgery

All (PTC + FTC)		WBS		Total
		+	–	
IS	+	32	5	37
	–	2	7	9
Total		34	12	46

**TABLE 4.** Results of  $^{131}\text{I}$  WBS uptake and IS with a monoclonal Ab against hNIS in 27 patients with PTC

PTC		WBS		Total
		+	–	
IS	+	16 (14)	3 (2)	19 (16)
	–	5 (2)	3 (2)	8 (4)
Total		21 (16)	6 (4)	27 (20)

Shown in parentheses are results for the same subgroup, excluding all patients on thyroid hormone at the time of surgery.

2) the presence of an, as yet, unidentified inhibitor of NIS activity, or 3) dedifferentiation of tumor cells within the metastatic or recurrent deposit compared with the primary tumor so that the pattern of NIS expression of the primary lesion does not accurately represent that of the metastatic deposit.

A recent study analyzing a large number of thyroid tumor samples revealed that hNIS transcriptional failure, as well as additional posttranscriptional mechanisms could account for loss of hNIS function, thereby resulting in loss of iodide-concentrating ability in these tumors (20). In fact, it was shown that methylation of certain regions of the hNIS promoter could lead to loss of hNIS expression, with resultant loss of uptake. Moreover, successful treatment of these tumors with chemical demethylation agents resulted in restoration of hNIS (20). Although the potential mechanisms that could affect the functional integrity of hNIS are many, ef-

fective RAI uptake requires more than a functional hNIS gene. A sufficient number of functional TSH receptors and normal downstream signal transduction pathways of TSH-stimulated hNIS expression are also necessary for adequate iodide uptake (20). Failure at any of these multiple levels could result in loss of iodide-concentrating ability by thyroid follicular cells. Determining which of these mechanisms are operational in our patients with a negative scan but positive IS would require further investigation.

Another potential reason for discrepancy between WBS and NIS IS results may have been inadequate preparation for WBS. However, adequate preparation by thyroid hormone withdrawal was documented by TSH measurements at the time of RAI scanning in most patients. Nine patients did not have TSH levels determined at the time of scan, because these patients underwent the procedure before the TSH assay was available. Of these nine patients, only two had negative WBS, and each of these patients were prepared for WBS by appropriate withdrawal of thyroid hormone 6 wk before the procedure. Therefore, inadequate preparation resulting in a falsely negative scan, although possible, is an unlikely explanation for the discordant results.

The current study is limited by its retrospective nature and the necessity to use archival, paraffin-embedded tissue specimens to achieve adequate numbers for evaluation. It is uncertain, although possible, that the use of archival material, which in some of our cases dates back to 1966, could lead to alteration of some antigenic sites that our antibody recognizes affecting its specificity. We did not observe that older samples were either more or less likely to demonstrate NIS IS. This question can only be answered by prospective studies analyzing freshly obtained tissue samples. However, the results do suggest that NIS IS of the thyroidal primary tumor in patients with PTCs and FTCs has substantial ability to predict the behavior of subsequent deposits of metastatic and recurrent cancer, with respect to iodine trapping and concentration. The findings require confirmation in prospective studies to determine more accurately

the predictive ability of the test and its role in the postoperative management of patients with DTC.

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Address all correspondence and requests for reprints to: John C. Morris, M.D., Mayo Clinic, Division of Endocrinology, 200 First Street Southwest, Rochester, Minnesota 55905. E-mail: Morris.John@Mayo.edu.

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