Differentiated Thyroid Carcinoma That Express Sodium-Iodide Symporter Have a Lower Risk of Recurrence for Children and Adolescents

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ABSTRACT

The sodium-iodide symporter (NIS) is expressed by papillary (PTC) and follicular (FTC) thyroid carcinoma, and is essential for iodine uptake. We hypothesized that PTC and FTC with detectable NIS immunostaining would be more amenable to radioactive iodine (^{131}I) treatment and follow a more benevolent course. To test this, we determined NIS expression by immunohistochemistry in 23 PTC, 9 FTC, and 12 benign thyroid lesions from children and adolescents. NIS expression was determined by two blinded examiners and graded as absent = 0, minimal = 1, moderate = 2, intense = 3, and very intense = 4. NIS was detected in 35% (eight of 23) of PTC, 44% (four of 9) of FTC, 25% (two of eight) of benign tumors, and 100% (four of four) of autoimmune lesions. The intensity of NIS expression was similar in PTC (0.61 ± 0.24), FTC (0.56 ± 0.24), and benign tumors (0.50 ± 0.33) but was more intense in autoimmune lesions (3.0 ± 0.7 , $p < 0.005$). Distant metastases were found only among PTC with undetectable NIS (two of 15, 13%), and recurrence developed exclusively from PTC and FTC with undetectable NIS (four of 20, 20% versus zero of 12, $p = 0.043$). The dose of iodine 131 required to achieve remission in the five patients with

PTC who had undetectable NIS (213.3 ± 53 mCi) was greater than that required by patients with similar age and extent of disease for whom NIS expression is unknown (109 ± 22 mCi, $p = 0.06$). We conclude that NIS expression is associated with a lower risk of recurrence for PTC and FTC of children and adolescents. (*Pediatr Res* 52: 737–744, 2002)

Abbreviations

NIS, sodium-iodide symporter
PTC, papillary thyroid carcinoma
FTC, follicular thyroid carcinoma
TTF-1, thyroid transcription factor-1
PAX-8, paired-domain transcription factor-8
GTP_α, stimulatory alpha subunit of guanosine triphosphate binding protein
ret/PTC, rearrangement of *ret* protooncogene
MACIS, metastasis-age-completeness-of-resection-invasion-size
Tg, thyroglobulin

The NIS is a membrane-bound glycoprotein cotransporter expressed by thyroid, salivary, gastric, mammary, and extraocular tissues (1–4). NIS is essential for the uptake of iodide and thyroid hormone synthesis and is directly related to the level of thyroid

differentiation (5–9). NIS expression is controlled by TSH and dependent on TTF-1 as well as PAX-8 (5, 10–15). Because of the central role of NIS in determining radioactive iodine uptake, it has been postulated that thyroid carcinoma that fail to express NIS would be resistant to radioactive iodine ablation (16).

In the normal thyroid, NIS expression is unequally distributed around individual thyroid follicles, and is most intense at the basolateral cell border (3, 14, 17–19). Such unequal NIS expression is also found at the macroscopic level, at which radioactive iodine scans reveal patchy uptake in thyroid tumors as well as in autoimmune thyroid disease (20). NIS is most highly expressed by Graves' tissue, in which a diffuse pattern of expression is characteristic (4, 21).

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A limited number of previous studies have used immunohistochemistry to examine NIS expression by thyroid carcinoma (4, 9, 21–25). A previous study by Jhiang *et al.* (21) failed to detect NIS in any of the four adult thyroid carcinomas examined. A second study by Saito *et al.* (24) found NIS expressed by seven of 17 PTCs. The patients ranged in age from 17 to 68 y, and 11 of 17 had metastases to the cervical lymph nodes, but none had distant metastasis. A study by Castro *et al.* (23) found intermediate staining for NIS in two adult FTCs and one adult PTC. Caillou *et al.* (4) examined four thyroid carcinomas from patients aged 21–77 y. They were also able to show that all three of the PTCs with positive NIS staining incorporated radioactive iodine. A recent abstract by Wapnir *et al.* (25) detected NIS in 71% of PTCs, 33% of FTCs, and 66% of benign thyroid tumors. Unfortunately, there were insufficient clinical details in any of these studies to determine whether NIS expression had an impact on clinical outcome. Lazar *et al.* (26) used quantitative reverse transcriptase-PCR to examine NIS mRNA levels in 43 thyroid carcinomas from patients aged 46.6 ± 13 y. NIS expression was lower in more advanced tumor stages. Using reverse transcriptase-PCR, Rinkel *et al.* (10) found reduced NIS mRNA levels in 90% of adult thyroid carcinomas. All these studies suggest NIS expression is common in differentiated thyroid carcinomas from adults, but the importance of NIS expression in defining clinical outcome remains unknown.

Most importantly, no previous study included any children or adolescents with thyroid cancer. This is important because thyroid cancers in children and adolescents are well-differentiated and therefore likely to express NIS. In addition, there are important clinical and molecular differences between thyroid cancers from children and adolescents compared with adults. First, thyroid cancers have a lower mortality rate but a higher recurrence rate in children and adolescents compared with adults (27–30). Although late recurrence can be seen, the vast majority of tumors that ever recur (90%) do so within the first 7 y after treatment (29). Second, disease-free survival is lower in adult FTC compared with PTC, but similar in PTC and FTC from children and adolescents (29). Finally, the pattern of oncogene expression also appears to differ. Thyroid cancers from children are less likely to harbor activating mutations in GTP_{src} , but more likely to have rearrangements in *ret*/PTC (31, 32). Because of these differences, it is important to understand the impact of NIS expression on the outcome of childhood thyroid cancers.

The current study was designed to determine NIS expression in a group of benign and malignant thyroid lesions from children and adolescents and to correlate NIS expression with the clinical outcomes of thyroid cancer. Our results are consistent with the hypothesis that NIS expression is a favorable prognostic indicator for thyroid carcinoma in children and adolescents.

METHODS

Approval. This study received prior approval from the Human Use Committee of the Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, DC, U.S.A.

Patients. The Automated Centralized Tumor Registry of the Department of Defense (ACTUR) was searched to identify all patients with differentiated thyroid carcinoma who were 21 y of age or younger at the time of diagnosis. Original medical records were used to construct a computerized database that includes demographic features, tumor characteristics, surgical treatment, adjunctive therapy, and clinical outcome. The extent of disease at diagnosis was classified according to the system of DeGroot *et al.* (33, 34) and the MACIS score. According to DeGroot *et al.* (33), class 1 disease was confined to the thyroid gland; class 2 involved the regional lymph nodes; class 3 either extended beyond the capsule or was inadequately resected; and class 4 had distant metastasis. Because all patients were younger than 39 y of age, MACIS scores were calculated as $3.1 + (\text{size} \times 0.3) + 1$ (if incomplete resection) + 3 (if distant metastasis) (34). As in previous studies, recurrence was defined as the appearance of new disease (identified by radioactive iodine scan or biopsy) in any patient who had been free of disease (no disease palpable or identified by radioactive iodine scan) for a period of 4 mo after initial therapy (29). Serum Tg levels were determined in contemporary patients (normal: 3–40 ng/mL, University of Southern California Clinical Laboratories, Los Angeles, CA, U.S.A.). However, not all historical patients had Tg levels available during the course of treatment. For this reason, serum Tg levels are not included in this definition, but are presented where available. The patients with PTC and FTC had normal or low TSH levels, and were clinically euthyroid at the time the primary tumors used in this study were obtained. The clinical details for some of the patients in this group have been previously published (29).

Formalin-fixed, paraffin-embedded archival tumor blocks corresponding with 23 of the PTC, nine FTC, and 12 benign thyroid lesions (six benign follicular adenomas, three Graves' disease, two multinodular goiters, one Hashimoto's thyroiditis) were available for study. For analysis, the benign lesions were separated into two categories (benign tumors and autoimmune lesions) and compared with PTC and FTC.

Immunohistochemistry. Sections from original, formalin-fixed, paraffin-embedded archival tissue blocks were sectioned and stained with hematoxylin and eosin to confirm the diagnosis (35). The sections immediately adjacent (5 μm) were used for immunohistochemistry. Sections were deparaffinized with xylene and rehydrated through a series of graded alcohol solutions followed by nuclease-free water. Endogenous peroxidase was inactivated (3% H_2O_2 , 30 min), and antigen was retrieved in citrate buffer (pH 6.0, 30 min, 100°C, steamer). Tissue sections were sequentially incubated with primary rabbit polyclonal NIS antibody (1:500, room temperature, 1 h) followed by detection using the Ventana Nexes automated immunostainer (Ventana Medical Systems, Tucson, AZ, U.S.A.) (21, 36, 37). The secondary antibody and ABC reagent were obtained from the Elite Vector Kit (Vector Laboratories, Burlingame, CA, U.S.A.) and used according to the manufacturer's suggestions. The addition of substrate chromogen (diaminobenzidine) was performed manually (10 min, room temperature) followed by hematoxylin counterstain (30 s). Sections of adult Graves' disease were used as the positive control, and PBS was substituted for the primary and second-

ary antibodies and used as the negative controls. The presence and intensity of NIS staining was determined by two blinded, independent examiners and graded as follows: 0 = absent, 1 = minimal, 2 = moderate, 3 = intense, and 4 = very intense. The interobserver agreement was >90%, and the three discordant slides were graded by a third examiner. The two scores in agreement were then used as the final intensity grade. For some data analyses, tumors were stratified into two groups: those in which NIS was detected (grade 1–4) and those in which NIS was not detected (grade 0). Of importance, the interexaminer concordance for this distinction was 100%.

Data analysis and statistical comparisons. The intensity of NIS expression for PTC, FTC, and benign lesions was then compared. The presence or absence of NIS immunostaining was also correlated with the demographic features, histologic variant, focality of the tumor, size of the tumor, extent of disease at diagnosis (class 1–4), MACIS score, and clinical outcome. Statistical analyses were performed using SPSS for Windows 95 (version 7.5, SPSS Inc., Chicago, IL, U.S.A.). The intensity of NIS expression was compared by ANOVA, correlations were performed using Pearson correlation, and nonparametric analyses were performed using either the χ^2 or Fisher's exact test as indicated.

RESULTS

The specificity of NIS staining was confirmed using a sample of adult Graves' disease as a positive control, and PBS for the negative controls. In adult Graves' tissue, NIS was diffusely evident in a generalized distribution as previously reported (Fig. 1A) (21). When PBS was substituted for the primary or secondary antibody, NIS staining was completely abolished. In addition, there was no effect of the length of time each tumor block had been stored on the intensity of NIS

staining ($r = 0.22$, $p = 0.30$). Representative NIS staining for each intensity category is also shown in Figure 1 (B, grade 0 = absent; C, grade 1 = minimal; D, grade 2 = moderate; E, grade 3 = intense; and F, grade 4 = very intense). Of note, NIS immunostaining of PTC and FTC was diffuse and cytoplasmic in location (Fig. 1, B–D) as reported for adults (21).

Demographic features and NIS immunostaining for individual cases of PTC and FTC are shown in Table 1. For patients with PTC, the average age was 16.1 ± 4.3 y (median, 16 y; range, 6–21 y), mean tumor size was 2.0 ± 1.5 cm (range, 0.5–7.5 cm), and the average follow-up was 48 ± 42 mo (range, 0–169 mo). Eight of the patients with PTC (35%) were followed more than 5 y, and four (17%) were followed more than 7 y. For patients with FTC, the average age was 18.3 ± 2.0 y (median, 19 y; range, 16–21 y), mean tumor size was 2.6 ± 1.4 cm (range, 0.2–4.0 cm), and the average follow-up was 39 ± 14 mo (range, 18–60 mo). None of the patients with FTC was followed beyond 5 y. The demographic features of these patients with PTC and FTC are similar to the larger series previously published by our group (29).

Figure 2 compares NIS staining for PTC, FTC, benign thyroid tumors, and autoimmune lesions. NIS staining was of similar intensity for all benign and malignant thyroid tumors (PTC, 0.61 ± 0.24 ; FTC, 0.56 ± 0.24 ; benign tumors, 0.50 ± 0.33). Overall, NIS was detected in two of eight benign tumors (25%, both benign adenomas), 35% of PTCs, 44% of FTCs, and 100% of autoimmune lesions. Three of four (75%) of the autoimmune lesions in this study were from children with Graves' disease. Because the most intense NIS expression has been found in adults with Graves' disease, we expected and found that mean staining intensity was significantly greater for childhood autoimmune thyroid lesions (3.0 ± 0.71) compared

Figure 1. Representative immunostaining for NIS. A, intense, diffuse staining characteristic of Graves' disease. B–F, representative examples of NIS staining: grade 0 (B, absent), grade 1 (C, minimal), grade 2 (D, moderate), grade 3 (E, intense), and grade 4 (F, very intense). All micrographs are shown at $\times 200$ magnification.

Table 1. Individual patient demographics and NIS expression

Age*/ gender	Tumor size (cm)†	MACIS‡	Class§	Surgery RAI¶	Recur (Y/N)	Time to recur (mo)	Individual NIS scores**		
							1st	2nd	3rd
NIS-positive papillary carcinoma									
20/F	1	3.4	2	T/Y	N		4	4	
11/F	2	3.7	1	?/N	N		1	1	
18/F	1.8	3.64	2	T/Y	N		1	1	
21/F	1.8	3.64	1	T/N	N		1	2	1
15/F	7.5	5.35	2	T/Y	N		1	2	1
16/F	1.5	3.55	1	L/Y	N		1	1	
18/F	1.5	3.55	1	T/Y	N		4	4	
17/M	2.6	4.88	3	T/Y	N		1	1	
NIS-positive follicular carcinoma									
17/M	NA	NA		?/?	N		1	1	
19/F	NA	NA		?/?	N		1	2	1
17/F	2.5	3.85		T/Y	N		2	2	
19/F	2.8	3.94		T/N	N		1	1	
NIS-negative papillary carcinoma									
6/F	4.3	7.39	4	NT/Y	Y	6	0	0	
21/F	2.3	3.79	1	T/?	N		0	0	
19/M	1	3.4	2	T/?	N		0	0	
18/F	2.9	3.97	1	T/Y	N		0	0	
21/F	1	3.4	2	T/?	N		0	0	
20/F	0.5	6.25	4	T/Y	N		0	0	
14/M	0.5	3.25	1	T/N	N		0	0	
15/F	2.4	3.82	1	T/Y	N		0	0	
19/M	1.1	3.43	1	ST/?	N		0	0	
6/F	2.6	3.88	1	T/?	N		0	0	
17/F	2.4	3.82	1	T/Y	N		0	0	
10/F	0.8	3.34	2	T/Y	N		0	0	
19/M	0.5	3.25	2	T/Y	N		0	0	
14/M	2.0	3.7	1	T/Y	Y	11	0	0	
15/M	NA	NA	3	?/Y	Y	40	0	0	
16/M	NA	NA	1	?/?	N		0	0	
NIS-negative follicular carcinoma									
16/F	4.0	4.3		T/Y	N		0	0	
21/F	4.0	4.3		ST/Y	N		0	0	
17/F	2.2	3.7		T/Y	Y	9	0	0	
20/F	0.2	3.16		T/Y	N		0	0	

* Patient age in years.

† Tumor size along the longest tumor axis.

‡ MACIS score = 3.1 + (size × 0.3) + 1 (if incomplete resection) + 3 (if distant metastasis) 34.

§ Class according to the classification system of DeGroot *et al.* 33 pertains only to PTC.

|| Surgery classified as total thyroidectomy (T), near-total (NT), subtotal (ST), lobectomy (L), or unknown (?) 29.

¶ RAI ablation given (Y) or not given (N).

** Individual NIS scores are shown with the reading of the third scorer where required.

NA, not available; RAI, radioactive iodine.

with PTC (0.61 ± 0.24 , $p = 0.001$), FTC (0.56 ± 0.24 , $p = 0.001$), and benign thyroid tumors (0.50 ± 0.33 , $p = 0.004$).

PTC and FTC were stratified into two groups: tumors with detectable NIS expression ($n = 12$), and tumors without detectable NIS expression ($n = 20$). As shown in Table 2, the extent of disease at diagnosis was similar when comparing the two groups, as were the average MACIS score and tumor size for both PTC and FTC. Of note, however, neither of the class 4 tumors in this study (PTC with distant metastasis) had detectable NIS. As shown in Table 2, the treatment received by patients with PTC or FTC who had detectable NIS was similar to that received by patients with PTC or FTC who had undetectable NIS.

Despite similar extent of disease at diagnosis, tumor size, and treatment, the overall recurrence risk was increased for tumors that had undetectable NIS expression. Recurrent dis-

ease developed exclusively from tumors in which NIS was not detected (four of 20, 20%, $p = 0.043$). The average time to recurrence was 16.5 ± 7.9 mo (range, 6–40 mo). This is shorter than the average time to recurrence for patients in our database with similar age and extent of disease at diagnosis, for whom NIS expression is unknown (45 ± 55 mo, $p = 0.18$; Table 3). However, the difference did not achieve statistical significance. Two patients with PTC had very intense (grade 4) NIS immunostaining. Both were young women (18 and 20 y of age) with unifocal, class 1 and class 2 disease. The PTC were small (1.0 and 1.5 cm), and MACIS scores were low (3.4 and 3.55). Neither patient had a recurrence, and at last follow-up, both were alive without disease.

Detailed records of radioactive iodine treatments were available for five of the patients with PTC who had undetectable

Figure 2. NIS expression among PTC, FTC, benign tumors, and autoimmune lesions. NIS expression was of similar intensity for benign and malignant tumors, but significantly more intense in autoimmune lesions.

NIS expression. Three of these patients had class 1 disease, one had class 2 disease, and one had class 4 disease (Table 3). Although four of five (80%) were eventually free of disease, the dose of radioactive iodine required to achieve remission (213.3 ± 53 mCi) was higher than the average received by patients in our database with similar age and extent of disease for whom NIS expression is unknown (109 ± 22 mCi, $p = 0.06$). Table 3 compares the radioactive iodine treatments received by these five patients stratified according to disease class. Patients with class 1 disease (confined to the thyroid) required significantly more radioactive iodine to achieve remission when NIS staining was absent (224 ± 56 versus 66 ± 16 , $p = 0.02$). There were too few patients with more extensive disease to allow independent statistical comparison between the class 2 and class 4 patients.

DISCUSSION

Expression of NIS is a marker of differentiated thyroid function, and at least some level of NIS expression is essential for successful therapy of thyroid carcinoma using radioactive iodine ablation (1–16). The utility of NIS immunostaining has been previously studied but only on a limited number of adult thyroid carcinomas. In these adult samples, NIS was detected by immunohistochemistry in several, but not all, cases (4, 9, 21–23). Unfortunately, none of these studies provided sufficient clinical details to determine whether NIS expression could be correlated with the response to therapy or the risk of recurrence. Furthermore, none of these studies included any significant numbers of children or adolescents. This latter

omission is important, because several findings indicate that thyroid carcinoma of children and adolescents follow a more benign clinical course and may have a different pattern of growth factor and oncogene activation (27–32). For these reasons, the current study was designed to examine NIS expression among benign and malignant thyroid tumors and to correlate NIS expression with the outcome of thyroid carcinoma in children and adolescents.

The data show that NIS is detected in similar proportions of benign (25%) and malignant (PTC, 35%; and FTC, 44%) thyroid tumors from children and adolescents ($p = 0.41$). This provides a potential explanation for the common observation that thyroid scintigraphy is generally unable to distinguish benign from malignant thyroid lesions (38). Our findings are consistent with previous studies of adult PTC, in which NIS was detected using immunohistochemistry in zero of four, seven of 17, three of three, and three of four adult PTCs (4, 9, 21, 22). Furthermore, NIS immunostaining of PTC and FTC was diffuse and cytoplasmic in location, as has been previously reported (4, 9, 21, 22). In our study, the patients with PTC and FTC had normal or low TSH levels during surgery. It is possible that some of the variability in published findings could relate to variability in serum TSH levels. During thyroid hormone withdrawal for the treatment of thyroid carcinoma with radioactive iodine, the resultant TSH elevation could increase NIS expression above the detection threshold in a larger proportion of tumors (5, 10–15).

NIS expression had a significant impact on the clinical course of individual patients in our study population. First,

Table 2. Clinical features and NIS expression for PTC and FTC

	NIS positive	NIS negative	p value
Total	n = 12 (37.5%)	n = 20 (62.5%)	
PTC/FTC	8/4	16/4	NS
Gender (M/F)	2/10	7/13	NS
Extent of disease at diagnosis (class)*			
Class 1 (PTC only)	4/8 PTC (50%)	9/16 PTC (56%)	NS
Class 2 (PTC only)	3/8 PTC (37.5%)	4/16 PTC (25%)	NS
Class 3 (PTC only)	1/8 PTC (12.5%)	1/16 PTC (6.25%)	NS
Class 4 (PTC only)	0	2/16 PTC (12.5%)	NS
MACIS†	3.96 ± 0.72	4.06 ± 1.17	NS
Tumor size (cm)	2.50 ± 1.84	1.92 ± 1.30	NS
Unifocal disease (%)‡	6 (60%)	11 (61%)	NS
Multifocal disease (%)‡	4 (40%)	7 (39%)	NS
Thyroid surgery			
Total thyroidectomy	8 (88%)	15 (83%)	NS
Near total thyroidectomy	0	1 (5.5%)	NS
Subtotal thyroidectomy	0	2 (11%)	NS
Lobectomy	1 (12%)	0	NS
Unknown	3	2	
Lymph node surgery			
Only suspicious nodes removed	3 (50%)	12 (67%)	NS
Modified radical neck dissection	2 (33%)	4 (22%)	NS
Radical neck dissection	1 (17%)	2 (11%)	NS
Unknown	6	2	
RAI ablation	7 (58%)	13 (65%)	NS
Follow-up (mo)	33 ± 31	53 ± 40	NS
Outcome			
Remission	6 (50%)	11 (55%)	NS
Persistent disease	4 (33%)	7 (35%)	NS
Recurrence	0	4 (20%)	0.043

* Class according to the classification system of DeGroot *et al.* 33 pertains only to PTC.

† MACIS score = 3.1 + (size × 0.3) + 1 (if incomplete resection) + 3 (if distant metastasis) 34.

‡ Focality indicates unifocal lesions or multifocal lesions based on microscopic examination.

RAI, radioactive iodine.

none of the PTC with distant metastases (lung or bone) at diagnosis had detectable NIS expression. Because NIS expression is a powerful indicator of thyroid differentiation, our data suggest the possibility of a subtle decrease in the level of differentiation among PTC that have acquired a metastatic phenotype (5–9). Second, tumors that failed to stain for NIS were more likely to recur ($p = 0.043$), despite the fact that all patients had similar extent of disease at diagnosis, similar tumor size, and similar treatment. Follow-up was slightly longer for patients who had tumors with undetectable NIS (53 ± 40 mo *versus* 33 ± 31 mo), but the difference was not statistically significant and all recurrent disease developed by 40 mo after diagnosis. Although late recurrence can be seen in childhood thyroid cancers, 90% of all recurrence develops in the first 84 mo after treatment (29). Although it is possible that some of these patients may go on to develop recurrent disease, the data support the hypothesis that thyroid tumors with detectable NIS expression have a reduced probability of recurrence.

Detailed radioactive iodine treatment records were available for five of the patients with PTC who failed to express NIS. Although four of five (80%) were eventually free of disease, the dose of radioactive iodine required to achieve remission (213.3 ± 53 mCi) was higher than the average received by patients in our larger database with similar age and extent of disease for whom NIS expression is unknown (109 ± 22 mCi,

$p = 0.06$). This difference was even more significant when comparing patients with class 1 disease (disease confined to the thyroid gland, 224 ± 56 mCi *versus* 66 ± 16 mCi, $p = 0.02$). Although NIS was not detected in this subset of tumors, they did respond to radioactive iodine ablation. All the tissues in this study were removed while the patients were clinically euthyroid, but NIS expression is exquisitely sensitive to TSH stimulation (5, 10–15). It is probable that during thyroid hormone withdrawal and the resultant TSH elevation used to prepare patients for radioactive iodine ablation, NIS expression would have been sufficiently increased to allow radioactive iodine uptake and successful treatment, albeit with increased doses.

The optimal treatment for PTC and FTC in children and adolescents is debated; however, both histologic variants have similar disease-free survival and have been similarly treated in children and adolescents (29, 30, 39). For that reason, our cases of PTC and FTC were analyzed together. PTC and FTC are commonly treated using annual doses of radioactive iodine until the patient is free from disease (30, 39). The patients in our study were not treated with a prospective protocol, but they all received annual radioactive iodine treatments until they were free from disease. Our data support the theory that carcinoma with detectable NIS expression are more amenable to treatment with radioactive iodine than are carcinoma with undetectable NIS. However, our data are limited by the small

Table 3. Radioactive iodine treatment of patients with PTC

	NIS negative	NIS unknown	<i>p</i> value
Total	<i>n</i> = 5	<i>n</i> = 26	
Gender (M/F)	2/3	8/18	NS
Extent of disease at diagnosis (class)*			
Class 1 (PTC only)	3	13	NS
Class 2 (PTC only)	1	9	NS
Class 3 (PTC only)	0	2	NS
Class 4 (PTC only)	1	2	NS
MACIS†	4.7 ± 1.8	4.14 ± 0.6	NS
Tumor size (cm)	2.8 ± 1.0	1.65 ± 0.18	NS
Unifocal disease (%)‡	3 (60%)	13 (50%)	NS
Multifocal disease (%)‡	2 (40%)	13 (50%)	NS
Thyroid surgery			
Total thyroidectomy	4 (80%)	18 (70%)	NS
Near total thyroidectomy	1 (20%)	0	NS
Subtotal thyroidectomy	0	7 (27%)	NS
Lobectomy	0	1 (4%)	NS
Unknown			
Lymph node surgery			
Only suspicious nodes removed	3 (60%)	7 (27%)	NS
Modified radical neck dissection	0	3 (11%)	NS
Radical neck dissection	0	1 (4%)	NS
None	1 (20%)	13 (50%)	NS
Unknown	1 (20%)	2 (8%)	NS
Cumulative dose to achieve remission (mCi, mean ± SEM)§	213 ± 53	109 ± 22	0.06
Stratified according to disease class			
Class 1	224 ± 56 (<i>n</i> = 3)	66 ± 16 (<i>n</i> = 13)	0.02
Class 2	62.4 (<i>n</i> = 1)	113 ± 24 (<i>n</i> = 9)	
Class 3	none were class 3	125 ± 25 (<i>n</i> = 2)	
Class 4	333.4 (<i>n</i> = 1)	350 ± 200 (<i>n</i> = 2)	
Follow-up (mo)	68.4 ± 61.7 (0–169)	99 ± 85 (16–332)	NS
Outcome			
Remission	4 (80%)	18 (70%)	NS
Persistent	0	3 (12%)	NS
Recurrent	3 (60%)	5 (19%)	0.09
Time to recur (mo)	16.5 ± 7.9	45 ± 55	0.18

* Class according to the classification system of DeGroot *et al.* 33 pertains only to PTC.

† MACIS score = 3.1 + (size × 0.3) + 1 (if incomplete resection) + 3 (if distant metastasis) 34.

‡ Focality indicates unifocal lesions or multifocal lesions based on microscopic examination.

§ Cumulative dose of ¹³¹I (mCi) required to achieve initial remission.

number of subjects, and by the retrospective nature of patient treatment and follow-up.

Serum Tg levels were available only on the contemporary patients. For this reason, we defined recurrence as the appearance of new disease identified by radioactive iodine scan or biopsy (29). It is possible that some of the historical patients who were believed to be free of disease might have had detectable serum Tg and could have been inappropriately categorized. Nevertheless, treatment of such scan-negative, Tg-positive patients is highly controversial (40). In addition, all contemporary patients who were designated free of disease had serum Tg values less than 2 ng/mL. Further study of NIS expression is clearly warranted. However, our data are consistent with the hypothesis that failure to detect NIS by immunostaining in tumors from euthyroid patients is associated with a requirement for greater radioactive iodine doses to achieve remission and an increased recurrence risk.

CONCLUSIONS

In conclusion, our data show that NIS expression is a favorable prognostic indicator for differentiated thyroid carcinoma of children and adolescents. The risk for recurrence is significantly increased for carcinoma from euthyroid patients in whom NIS cannot be detected by immunostaining, and the doses of radioactive iodine required to achieve remission are larger. Additional study is required to validate these findings on a larger and more diverse patient population, but the data suggest the possibility that determination of NIS expression by immunohistochemistry could be used to stratify treatment and prognosticate for individual patients.

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