ORIGINAL ARTICLE

Methylation Status of Genes in Papillary Thyroid Carcinoma

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Objectives: To determine the methylation status of gene promoter regions using methylation-specific polymerase chain reaction in genes encoding for thyrotropin receptor (*TSHR*), E-cadherin (*ECAD*), sodium iodide symporter protein (*NIS-L*), ataxia telangiectasia mutated (*ATM*), and death-associated protein kinase (*DAPK*) proteins and if methylation status correlates with patient variables, tumor factors, or outcome measures among patients with papillary thyroid carcinoma.

Design: Database query and retrospective medical chart review for patients with well-differentiated thyroid cancer and nonmalignant thyroid conditions treated at our institutions (1996-2004). Methylation-specific polymerase chain reaction was performed, and results were compared with controls for these genes. Methylation status was then compared with patient variables, tumor factors, and outcome measures for patients with thyroid carcinoma and controls.

Patients: The study population comprised 32 patients with papillary thyroid carcinoma and 27 controls.

Results: In our patients, all 5 genes were methylated more frequently in papillary thyroid carcinoma than in controls. *NIS-L* trended toward a more advanced stage at presentation. *NIS-L* methylation in cancer cells was not associated with methylation in adjacent benign tissue, unlike the other 4 genes. Neither age nor sex affected methylation status, and methylation status did not correlate with extent of the primary tumor or presence of nodal metastasis at diagnosis. Tumors recurred less frequently in patients with *TSHR* methylation than in patients with unmethylated *TSHR* promoter regions.

Conclusions: Promoter methylation may be a marker for malignancy in thyroid carcinoma. Furthermore, methylation status of tumors as determined by methylation-specific polymerase chain reaction may help in determining patient prognosis.

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THYROID CARCINOMA IS A common malignant neoplasm that accounts for more than 90% of endocrine malignant tumors and results in 33 500 new cases and 1500 deaths annually in the United States.[1] Papillary and follicular thyroid carcinomas are also referred to as well-differentiated thyroid cancers and typically portend a favorable prognosis. However, clinicians treating patients with thyroid cancer face a number of challenges. One such challenge is the ability to make the correct diagnosis. Fine- needle aspiration (FNA) has become the standard of care for evaluating a thyroid nodule, but FNA and even intraoperative frozen section can be inconclusive in distinguishing a malignant from a nonmalignant lesion. Additional diagnostic modalities need to be developed to increase the sensitivity of cytologic and frozen histologic examination in formulating the diagnosis of well-differentiated thyroid carcinoma. Another challenge for physicians is determining which patients with well-differentiated thyroid carcinoma have a poor prognosis. The American Joint Committee on Cancer (AJCC) staging system (**Figure 1**) identifies patient age, primary tumor size and extent, presence of nodal disease, and presence of distant metastasis as markers for prognosis.[2] However, we have limited knowledge of which factors affect tumor behavior in histologically similar tumors. For example, patients older than 45 years have a worse outcome compared with younger patients despite similar tumor histologic type. There is a paucity of studies that correlate biological or molecular tumor characteristics with clinical outcome. One final challenge for treating physicians is that well-differentiated thyroid cancer is not responsive to standard chemotherapeutic agents or external beam radiation.[3] Treat-

ment with radioactive iodine (^{131}I) has been shown to have a positive impact on the survival of patients with well-differentiated thyroid carcinoma, but there is a subset of these tumors that do not exhibit significant uptake of iodine. Treatment of patients with these noniodine avid tumors presents a therapeutic challenge.

Recent research involving thyroid cancer has been extended to the molecular level. Unlike the *RET* proto-oncogene mutations for medullary thyroid carcinoma to date, 1 dominant genetic alteration has not been identified for papillary thyroid carcinoma.[4] There have been a number of recent discoveries regarding the expression of genes in thyroid pathologic conditions. Specifically, it has been shown that messenger RNA (mRNA) levels coding for the sodium iodide symporter, thyrotropin receptor, thyroid peroxidase, and thyroglobulin are decreased in thyroid malignant tumors when compared with controls.[5] Although activating mutations have been demonstrated in toxic adenomas and papillary thyroid carcinoma, no specific inactivating mutations have been identified in well-differentiated thyroid cancers.[6-11] The lack of inactivating mutations indicate that another molecular process such as DNA methylation is likely responsible for the gene silencing and resultant reduction in expression. Aberrant DNA methylation has been demonstrated to inactivate tumor suppressor genes in numerous malignant tumors.[12-15] There has been recent work demonstrating methylation of promoter regions for the *NIS*, *TSHR*, and *ECAD* genes in thyroid malignant tumors.[16-18] All 3 of these genes have been found to be methylated more frequently in well-differentiated thyroid cancer than in benign controls.

In the present article, we report our results with methylation-specific polymerase chain reaction (MSP-PCR) for the promoter regions of genes encoding for the sodium iodide symporter (*NIS-L*), the thyrotropin receptor (*TSHR*), E-cadherin (*ECAD*), ataxia telangiectasia mutated (*ATM*), and death-associated protein kinase (*DAPK*) proteins. These genes were chosen because of their role in thyroid function and reports of relationships of their methylation status in thyroid and other cancers. The methylation status of the *NIS* and *TSHR* promoter regions are important for multiple reasons. These genes are specific to thyroid tissue and possibly play an important role in the uptake of iodine and normal cellular function.[5,16,17,19] Methylation of these gene promoters may decrease their expression and interfere with the ability to concentrate iodine, rendering ablative doses of ^{131}I ineffective. The *TSHR* promoter region has been reported to be methylated in 59% of patients with papillary thyroid cancer and was not methylated in any controls in one preliminary report.[17] Sodium iodide symporter mRNA expression has been shown to be decreased in multiple trials, and this has been proposed to be secondary to methylation of the promoter region.[5,16] Within the promoter region for the *NIS* gene, there are 2 regions at which methylation status has been studied and reported. These are the *NIS-L* and *NIS-C* regions. We have limited our evaluation to the *NIS-L* region owing to previous reports and our experience. In one report, low levels of mRNA encoding for the sodium iodide symporter protein correlated with a higher presenting stage of thyroid papillary carcinoma.[5] *ECAD* promoter methylation has been demonstrated in non–small cell lung carcinoma, bladder carcinoma, and prostate carcinoma.[20] This gene is an invasion and metastasis suppressor, and in one report, methylation of its promoter region was described in 83% of papillary carcinomas.[16] The *ATM* gene is another gene that has been shown to demonstrate promoter methylation in breast cancer.[21,22] The *ATM* gene regulates the cell cycle by phosphorylating both *p53* and *BRCA-1*, which halts the cell cycle when DNA damage has occurred.[23] *DAPK* is a proapoptotic gene that modulates cell death and is another gene for which the promoter has been shown to be methylated in certain malignant tumors. These malignant tumors include laryngeal squamous cell carcinoma, leukemia, lung carcinoma, prostate carcinoma, myeloma, and gastric carcinoma. Specifically, the promoter region for *DAPK* was shown to be methylated in 67% of laryngeal malignant tumors and strongly correlated with the presence of nodal metastasis.[24] In our review of the literature, there has been no evaluation of methylation status of *DAPK* or *ATM* with thyroid carcinoma.

AJCC Stage Grouping
for papillary and follicular thyroid carcinoma

	Age < 45 y		
Stage I	Any T	Any N	M0
Stage II	Any T	Any N	M1
	Age ≥ 45 years		
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1-3	N1a	M0
Stage IVA	T4a	N0	M0
	T4a	N1a	M0
	T1-4a	N1b	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Figure 1. American Joint Committee on Cancer (AJCC) staging for thyroid carcinoma.[2]

In the present study, we sought to determine the promoter methylation status of patients with papillary thyroid carcinoma and compare these results with the control patients with nonmalignant disease. The presence of methylated promoter regions in thyroid malignant tumors, which are novel and not found in controls, could identify possible genetic markers for the differentiation of malignant nodules. Once the methylation status of these genes was determined, we compared methylation status with variables including presenting stage, presence of nodal metastasis, presenting age, tumor recurrence, and extent of the primary tumor to see if there was any correlation between the down-regulation of genes by promoter methylation and clinical variables. The use of demethylating agents has been shown to restore decreased iodine uptake to cells with methylated promoter regions in vivo. If methylation of these genes are indeed a marker of virulence or poor response to treatment, the concept of using demethylating agents such as 5-azacytidine or sodium butyrate may prove promising.

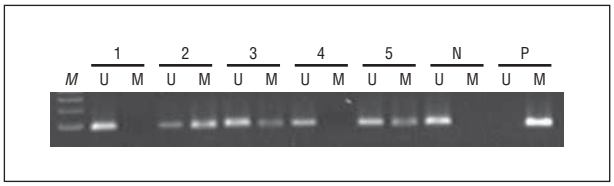

Figure 2. Methylation-specific polymerase chain reaction MSP-PCR analysis of *TSHR* promoter hypermethylation in 5 papillary thyroid carcinoma specimens. Both unmethylated- (U) and methylated-specific (M) MSP-PCR primer sets for *TSHR* were used. Negative (N; human placental DNA) and positive (P; universal methylated human DNA; Intergen Co, Purchase, New York) controls were included. The italic "M" (far left on the panel) indicates the molecular marker.

METHODS

MEDICAL CHART REVIEW

A total of 180 patients were identified as having been treated for their thyroid cancer at the University of Arkansas for Medical Sciences and the John McClellan Veterans Administration Hospital, Little Rock, between 1996 and 2004. Of the 180 patients, those who had a thyroidectomy at another institution prior to entry into our health care systems were not included because their pathologic specimens were not available. The patient medical charts were reviewed, and pertinent demographics and clinical and pathologic data were recorded. Of these 180 patients, 94 underwent surgical resection at our institution, with 107 pathologic specimens available for review (some patients had multiple procedures or multiple specimens from a single procedure). In addition, there were 27 patients with nonmalignant disease, including 10 follicular adenomas, 15 goiters, and 2 normal thyroids, who were identified for the control group. All of these tissue blocks were reviewed by a head and neck pathologist (C.-Y.F.) for histologic confirmation prior to MSP. To date, we have performed MSP-PCR on the 32 patients with papillary thyroid carcinoma who had a primary tumor specimen available and on the 27 controls.

METHYLATION-SPECIFIC PCR

After the tissue diagnosis was confirmed, microdissection and MSP-PCR were conducted. DNA samples were collected from tissue of the 59 patients. Samples were obtained from the lesions, histologically benign tissue adjacent to the malignant tumors, and from lymph node metastasis when available. DNA samples were collected using the EX-WAX DNA Extraction Kit (Intergen Co, Purchase, New York) according to the manufacturer's protocols. The DNA was then treated with sodium bisulfite to convert cytosine to uracil as follows. Treated DNA (2 µg in 20 µL of water containing 5 µg of salmon sperm DNA) was denatured by incubation in 0.3M sodium hydroxide at 50°C for 20 minutes. The DNA was incubated in a 500-µL reaction mixture containing 2.5M sodium metabisulfite and 0.125M hydroquinone (pH 5.0) at 70°C for 3 hours. Human placental DNA was used as a negative control, and CpGenome universal methylated human DNA (Intergen Co) served as a positive control. Amplification of the promoter region of the genes was carried out in a Touchgene Gradient Thermal Cycler (Techne Inc, Princeton, New Jersey) using Qiagen Hotstar Taq Kit (Qiagen, Valencia, California) in a 50-µL PCR mixture containing 4 µL of bisulfite-treated genomic DNA (Operon Technologies Inc, Alameda, California). The PCR conditions were as follows: initial denaturation and hot start at 95°C for 15 minutes, then 40 cycles consisting of 30 seconds at 95°C, 30 seconds at 68°C, and 1 minute at 72°C, followed by a final 5-minute extension at 72°C. Positive and negative control DNA samples and controls without DNA were used for each set of reactions. The final PCR products were resolved with agarose gel electrophoresis and stained with ethidium bromide (**Figure 2**). Both methylated and unmethylated primers were used for each gene and are listed for reference in **Table 1**.

This MSP-PCR was qualitative PCR; therefore, specimens with detectable methylated promoter regions were considered to be methylated, while those without methylated regions on PCR analysis were deemed unmethylated. Gene-specific methylation status was compared in the papillary thyroid cancer and control specimens. With the help of our biostatistician, a Fisher exact test was used to compare the number of papillary thyroid cancer and control specimens with methylated promoters for each gene. Each patient's presenting stage (Figure 1) was recorded, and the presenting stages for patients were compared (papillary thyroid carcinoma group vs control group) for each gene using a Fisher exact test. A Fisher exact test was also used for the remainder of our comparisons including age comparisons, primary tumor stage, presence of nodal disease, and presence of recurrence during follow-up. When the subgroups of malignant tumors with methylated promoters were examined to see if adjacent tissue was also methylated, the number of specimens was not sufficient to make statistical comparisons.

RESULTS

The demographics of the patients with thyroid cancer were compared with the control group. The mean age of the patients with thyroid cancer was 50.2 years compared with 60.0 years in the control group. The control group comprised 3 women and 24 men; and the thyroid cancer group, 13 women and 19 men. These differences were both statistically significant ($P=.03$ [age] and $P=.009$ [sex]). Our cancer population was younger as a group and consisted of more women compared with our controls.

To determine if any of the genes under study were more consistently methylated in the cancer specimens than in the controls, gene-specific methylation status was compared between the papillary thyroid cancer specimens and controls (**Table 2**). The 4 genes (*NIS-L*, *ATM*, *ECAD*, and *TSHR*) were found to be methylated in malignant tissue more often than in the controls, and this difference was significant for all 4 genes ($P<.05$). In all controls (nonmalignant), the promoter regions for *NIS-L*, *ATM*, and *ECAD* were unmethylated. Two of the adenoma specimens had methylated promoter regions for the *TSHR*, the remainder of the 25 controls were negative for *TSHR* promoter methylation. This was significantly different from the 11 of 32 papillary carcinoma specimens in which *TSHR* was methylated ($P=.12$ for *NIS-L*, $P<.001$ for *ATM*, $P<.001$ for *ECAD*, and $P=.03$ for *TSHR*). Early in our experiments, we did not obtain reliable data for *DAPK* MSP, so the methylation status for *DAPK* was not determined in the control group or the first 10 papillary specimens.

The methylation status of all the tested genes was correlated with the stage of disease at presentation. To make this comparison, malignant specimens were stratified by methylation status for each gene and then correlated with AJCC stage for each group. The presenting stage for patients with methylation-positive *NIS-L* promoter regions in malignant specimens was higher than in patients with methylation-negative malignant tissue;

Table 1. Primers Used for Methylation-Specific Polymerase Chain Reaction

Gene	Primer Sequences	Temperature, °C
TSHR		
Unmethylated	5′-TGT AGA GTT GAG AAT GAG GTG ATT TT-3′ (sense); 5′-CAC CAA CTA CAA CAA ATC CAC CA-3′ (antisense)	53
Methylated	5′-TGT AGA GTT GAG AAT GAG GTG ATT TC-3′(sense); 5′- CAA CTA CAA CAA ATC CGC CG-3′ (antisense)	57
NIS-L		
Unmethylated	5′-TAG GAT AGA TAG ATA GTA GGG GTG GAT-3′ (sense); 5′-CTC CAC AAC CTC CAT AAA AAC AAA TAC A-3′ (antisense)	58
Methylated	5′-ATA GAT AGA TAG TAG GGG CGG AC-3′ (sense); 5′-GAC CTC CAT AAA AAC GAA TAC G-3′ (antisense)	58
ATM		
Unmethylated	5′-GTT TTG GAG TTT GAG TTG AAG GGT-3′ (sense); 5′-AAC TAC CTA CTC CCA CTT CCA A-3′ (antisense)	60
Methylated	5′-GGA GTT CGA GTC GAA GGG C-3′ (sense); 5′-CTA CCT ACT CCC GCT TCC GA-3′ (antisense)	60
DAPK		
Unmethylated	5′-GGA GGA TAG TTG GAT TGA GTT AAT GTT-3′ (sense); 5′-CAA ATC CCT CCC AAA CAC CAA (antisense)	60
Methylated	5′-GGA TAG TCG GAT CGA GTT AAC GTC-3′ (sense); 5′-CCC TCC CAA ACG CCG A-3′ (antisense)	60
ECAD		
Unmethylated	5′-TAA TTT TAG GTT AGA GGG TTA TTG T-3′ (sense); 5′-CAC AAC CAA TCA ACA ACA CA-3′ (antisense)	58
Methylated	5′-TTA GGT TAG AGG GTT ATC GCG T-3′ (sense); 5′-TAA CTA AAA ATT CAC CTA CCG AC-3′ (antisense)	60

Table 2. Methylation of Papillary Thyroid Carcinoma in Patients vs Controls

Gene	Promoter Methylation, % (No./Total No.) (Patients With Carcinoma)	Promoter Methylation, % (No./Total No.) (Controls)
NIS-L	22 (7/32)	0 (0/27)
ATM	50 (16/32)	0 (0/27)
ECAD	56 (18/32)	0 (0/27)
TSHR	34 (11/32)	7 (2/27)

Table 3. Comparison of Presenting Age of Patients for Methylated vs Unmethylated Tumors[a]

Gene	Mean Presenting Age of Patients With Methylated Tumors, y	Mean Presenting Age of Patients With Unmethylated Tumors, y
NIS-L	54	49
DAPK	53	46
ATM	52	48
ECAD	49	51
TSHR	55	48

[a] There was no statistically significant difference found for any gene.

however, this difference did not reach significance using a Fisher exact test ($P=.10$). This was the only gene that showed a difference in presenting stage between the methylated and nonmethylated specimens. No difference in presenting stage was appreciated for the 4 remaining genes.

Methylation status of the malignant tumors was compared with adjacent histologically benign thyroid tissue for each specimen to determine if the methylation status of the malignant tumor paralleled the surrounding thyroid tissue, or if the methylation status was a genetic alteration found only in the malignant tissue. We were able to compare tumor methylation to adjacent histologically benign tissue for *NIS-L*, *ATM*, *ECAD*, and *TSHR* in 27 of the 32 thyroid cancer specimens. In the other 5 specimens, the cancer composed the bulk of the specimen, making isolation of histologically benign tissue impossible. In the subpopulation that had undergone *DAPK* testing, 19 of the 22 specimens were available for comparison. For *NIS-L*, 5 of the 27 malignant specimens had promoter methylation, of which none had methylation of adjacent histologically benign tissue. Owing to the limited number of patients, no statistically significant conclusions could be drawn for this *NIS-L* comparison. No difference was noted in the methylation status of the cancer when compared with adjacent tissue for the remainder of the genes, indicating that the methylation status was comparable between the adjacent histologically benign thyroid tissue and the histologically malignant tissue for *ATM*, *ECAD*, *DAPK*, and *TSHR*.

Age and sex were examined with regard to methylation status of the malignant specimens. The 32 patients with thyroid cancer were divided into 2 groups, those younger than 45 years (n=14) and those who were 45 years or older (n=18). Forty-five years was chosen as the age for comparison because it is defined by the AJCC as the age above which prognosis declines (Figure 1). The total number of methylated genes was compared between the groups and found to be similar ($P=.26$). The patients' presenting age was compared for methylated vs unmethylated specimens for each gene (**Table 3**). There was no difference in the age of patients with a methylated specimen when compared with an unmethylated specimen for any of the genes. The number of methylated genes for the 18 men was not statistically different compared with the number for the 14 female patients.

The extent of the primary tumor (T stage as defined by the AJCC) and the presence or absence of nodal metastasis at presentation were compared between the methylated and unmethylated specimens for each gene. There

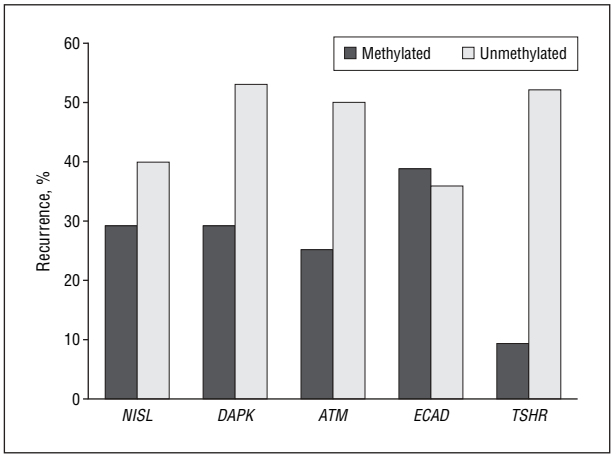

Figure 3. Comparison in recurrence between methylated and unmethylated tumors for each gene.

was no difference noted in the extent of the primary between methylated and nonmethylated tumors for any of the genes. For *NIS-L*, 57% (4 of 7) of the methylated specimens presented with nodal metastasis, and 24% (6 of 25) of the unmethylated specimens had nodal disease. However, this difference was not statistically significant ($P=.17$).

The presence or absence of recurrence was recorded for each patient. Recurrence was defined by the presence of clinically apparent and biopsy-proven disease or a thyroglobulin level greater than 2.0 ng/mL (to convert to micrograms per liter, multiply by 1.0) after thyroidectomy, ^{131}I ablation, and a history of undetectable thyroglobulin levels. The mean follow-up after presentation was 2.6 years (3 months to 8 years). The presence or absence of recurrence was compared between methylated and unmethylated groups for each gene (**Figure 3**). There was no statistical difference in recurrence between the groups for *NIS-L*, *DAPK*, *ATM*, or *ECAD*. Of the 21 patients, tumors in 11 with unmethylated promoter regions for *TSHR* recurred, and a tumor recurred in only 1 of the 11 patients with methylated *TSHR* promoter regions. This was a statistically significant difference ($P=.02$), indicating that in our population, tumors recur less in those with methylated promoter regions.

COMMENT

DNA promoter methylation is a known mechanism for the down-regulation of genes. Down-regulation of a protein that is associated with the suppression of tumorigenesis contributes to the development of malignant tumors. In this study, 5 genes were identified that have methylated promoter regions in a percentage of patients with papillary thyroid cancer. The methylation status of the controls for 4 of these genes, *NIS-L*, *ATM*, *ECAD*, and *TSHR*, was identified. In all 4 of these genes, the methylation status of patients with papillary thyroid cancer was more often methylated than in the controls with nonmalignant disease, which was statistically significant ($P=.12$ for *NIS-L*, $P<.001$ for *ATM*, $P<.001$ for *ECAD*, and $P=.03$ for *TSHR*). What makes this finding compelling is that methylation of the genes for *NIS-L*, *ATM*, or *ECAD* was only found in papillary thyroid cancer tissue, and none of the 27 controls had methylation of these genes. The finding that DNA methylation is found at such a distinctly different rate in papillary thyroid cancer than in nonmalignant specimens indicates that MSP-PCR holds promise from a diagnostic standpoint. The true diagnostic dilemma is distinguishing malignant from nonmalignant follicular lesions. We plan to continue to compile our data to include follicular carcinomas and follicular adenomas to explore the utility of considering MSP-PCR as a diagnostic tool as a supplement to FNA and intraoperative tissue sampling. For the *TSHR* gene, only 2 of the 27 controls had positive methylation status, and these were in 2 patients with follicular adenomas. There was no methylation of the *TSHR* promoter in benign or goitrous specimens. Some believe that there is a continuum of disease from benign thyroid tissue to follicular hyperplasia, follicular adenoma, and ultimately follicular thyroid carcinoma. There has been 1 report that showed a progressive increase in genetic alterations in each of these 4 steps.[23] One hypothesis that warrants further review is that DNA methylation accumulates in a similar manner and that promoter regions are methylated more frequently in adenomas than in benign tissue and even more yet in carcinomas. With our limited number of patients, this hypothesis cannot be corroborated, but it raises questions that can be evaluated further. We found that 32% of the promoter regions for *DAPK* were methylated, but we were not able to perform MSP-PCR on controls for comparison.

The results with the sodium-iodide symporter methylation status were intriguing. Although the difference was not statistically significant, tumors with positive methylation status for *NIS-L* had a more advanced presenting stage compared with those with negative methylation status. This correlates with previous reports that more advanced tumors at presentation had decreased expression of mRNA for the *NIS* gene.[5] Although delay in presentation and patient variables cannot be completely excluded, our results and those present in previous reports indicate that *NIS-L* methylation and decreased expression may be markers of virulence. Another interesting finding with the *NIS-L* gene is that the adjacent tissue was not methylated in any of the specimens we had available for comparison. This raises the following question: is methylation of this promoter region a late event in tumorigenesis? In the other 4 genes, methylation was found in most of the adjacent specimen, indicating that these epigenetic changes are found in the surrounding tissue that is histologically benign. These may represent field cancerization as described in other malignant tumors and supported by the prevalence of multifocal thyroid carcinomas. With the limited number of patients reported herein, statistical comparisons could not be made; however, we are continuing to assess tumor specimens and will be able to see if these preliminary findings are upheld.

Our cancer cohort differed from the control population in 2 demographics. The control population was older than the patients with papillary carcinoma by 10 years on average. There were more women among the patients with cancer than among the controls. In this study, neither age nor sex made a statistically significant dif-

ference in the number of methylated genes or in the methylation status of any specific gene.

One interesting concept is how alteration of genes coding for the uptake of iodine affect outcome in patients with well-differentiated thyroid carcinoma. It is expected that if genes controlling iodine uptake were down-regulated by methylation, the cancer would be more difficult to treat. If the tumor does not concentrate ^{131}I, one would expect these patients to have a worse outcome with more recurrences. However, our results to date do not support this. There was no difference in recurrence rate between patients with methylated and unmethylated *NIS-L*, and we actually found that tumors recurred less in patients with methylated *TSHR*. As we continue to collect data on our patients and learn more about how DNA methylation affects their clinical course, we plan to correlate methylation status of *TSHR* and *NIS-L* with iodine uptake in vivo. If a statistically significant decrease in ^{131}I uptake is demonstrated in these patients, methylation status could be used to predict the response to ^{131}I ablation. It has been shown that the addition of demethylating agents can restore iodine uptake in vitro for cells with methylated *NIS-L*.[14] It is possible that demethylating agents may play a role in redifferentiation of thyroid carcinomas in the future.

In conclusion, methylation of *NIS-L*, *TSHR*, *ECAD*, and *ATM* is more prevalent in patients with papillary thyroid carcinoma than in controls with nonmalignant disease. This difference is notable enough that it holds promise as a marker for malignant neoplasms in the future. Methylation of *NIS-L* trended toward an increase in AJCC stage at presentation. When *NIS-L* was methylated in papillary thyroid carcinoma, it was not methylated in adjacent histologically benign thyroid tissue. For the other 4 genes, the methylation status of the tumor was similar to that of the surrounding histologically benign tissue. Age and sex do not correlate with methylation of these particular genes in patients with papillary thyroid carcinoma. *TSHR* methylation was associated with decreased recurrence for our patients.

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Author Contributions: Dr Kokoska had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Fan, Bodenner, and Kokoska. *Acquisition of data:* Smith, Fan, and Zou. *Analysis and interpretation of data:* Smith, Fan, Zou, Bodenner, and Kokoska. *Drafting of the manuscript:* Smith and Kokoska. *Critical revision of the manuscript for important intellectual content:* Fan, Zou, Bodenner, and Kokoska. *Statistical analysis:* Smith. *Administrative, technical, and material support:* Smith, Zou, and Kokoska. *Study supervision:* Fan, Bodenner, and Kokoska.
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