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Higher rate of BRAF mutation in papillary thyroid cancer over time: A single institution study

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Abstract

Background—The incidence of thyroid cancer has doubled over the last decade. The reason for this dramatic increase in incidence is controversial. Some investigators have suggested the increased incidence is due to increased detection of small primary tumors as a result of diagnostic scrutiny. On the other hand, some investigators have demonstrated an increased incidence across all tumor sizes suggesting other factors may play a role. To investigate the clinical, pathologic and molecular changes present in papillary thyroid cancer over a 15 year period during which the incidence of papillary thyroid cancer doubled.

Methods—628 patients with conventional papillary thyroid cancer and 228 tumor samples from a single institution were analyzed from 1991 to 2005. Time-trend analyses of demographic, clinical, pathologic, and tumor genotype were performed over three 5 year time periods; Group I (1991–1995), Group II (1996–2000), and Group III (2001–2005).

Results—We found no differences in age, gender, ethnicity, primary tumor size, rate of extrathyroidal invasion, and overall TNM cancer stage among the three time groups. The rate of BRAF V600E mutation was significantly higher in Group III (88% BRAF V600E positive) as compared to Group I and II (51% and 43%, respectively) ($p < 0.001$). The rate of all the common somatic mutations was also significantly higher in Group III (92% positive) as compared to Group I and II (68% and 64%, respectively) ($p < 0.002$).

Conclusions—The rate of BRAF V600E mutation increased significantly over a 15 year period at our institution. Our findings suggest a higher rate of BRAF mutation in papillary thyroid cancer may contribute to the increasing incidence of thyroid cancer.

INTRODUCTION

The incidence of thyroid cancer has increased over the last decade; an increase of 2.4% per year in 1980–1997 to 6.5% per year in 1997–2006 (1). This increase is due mainly to the rise in papillary thyroid cancer, whereas the incidence of other types of thyroid cancer remains unchanged or is decreasing (1–3). The reason for the dramatically increasing incidence is controversial. Some researchers have attributed this trend to heightened medical surveillance

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All the authors had full access to all of the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis

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and improved diagnostics approaches such as high resolution thyroid ultrasound and fine needle aspiration biopsy (3). Because ultrasound can detect thyroid nodules as small as 0.2 cm in size this may result in increased detection of small thyroid cancer which usually do not have important clinical significance on morbidity and mortality. Also, increased detection of occult papillary thyroid cancer on pathologic examination of thyroid glands removed for benign conditions and reported as thyroid cancer cases to national cancer registries may account for the increasing incidence of thyroid cancer (4). Other investigators have found that the incidence rates for thyroid cancers of all tumor sizes has increased over time suggesting that diagnostic scrutiny is not the sole explanation (1,2). The increased incidence in thyroid cancer is seen among different countries and continents, and does not appear to depend on the relative rate of thyroid cancer (5–8). Thus, the increased incidence of thyroid cancer may represent a true increase in disease burden as a result of unrecognized environmental and dietary factors, or genetic changes.

Papillary thyroid cancer is the main histologic subtype of thyroid cancer and accounts for approximately 85% of all thyroid cancer diagnosis. About two-thirds of papillary thyroid cancers harbor somatic genetic alterations in the effectors of the mitogen-activated protein kinase signaling cascade. These effector genes include *RET/PTC* or *NTRK1* rearrangements and *RAS* or *BRAF* point mutations, and usually occur as mutually exclusive event in thyroid carcinogenesis (9).

To better understand the increasing incidence of thyroid cancer and the possible molecular basis, we investigated the clinical, pathologic and molecular changes present in 228 cases of conventional papillary thyroid cancer over a 15 year period during which the incidence of thyroid cancer doubled.

METHODS

Study Cohort and tumor samples

Six hundred twenty-eight consecutive patients with conventional papillary thyroid cancer and 228 primary tumor samples were analyzed from 1991 to 2005. All patients underwent initial evaluation and treatment at a single institution. In all cases, thyroidectomy was performed as treatment for their thyroid cancer and tissue from the primary tumor was procured in 228 of the 628 patients who agreed to have their tumor tissue used for research. Tumor tissue (approximately, 40 – 220 milligrams) was snap frozen immediately after removal. Tissue was procured for research use after informed consent. The study was reviewed and approved by the local institution review board.

Molecular testing for somatic genetic changes

Before molecular testing in each tumor sample, an endocrine pathologist re-reviewed each tumor sample to confirm it was conventional/classic papillary thyroid cancer (not follicular variant of papillary thyroid cancer or other variants of differentiated and poorly differentiated thyroid cancer) and contained ≥ 80% of tumor. Deoxyribonucleic acid (DNA) was extracted from frozen tumor tissue using DNA STAT-60 (Friendswood, TX) according to the manufacturer's instruction. DNA quantification and quality was assessed with a NanoDrop spectrophotometry and the 260/280 ratios ranged from 1.6 – 2.1. To further assess DNA quality in the tumor samples, we performed quantitative PCR for thyroglobulin using TaqMan Assay (Applied Biosystems) on an ABI 7900 HT system. Human GAPDH was used as an endogenous control. The primary dominant papillary thyroid cancer samples were genotyped for the following mutations: BRAF V600E point mutation, RET/PTC1, RET/PTC3 and NTRK1 rearrangements, and hotspot point mutations in KRAS and NRAS (codons 12/13 and 61). Point mutations in BRAF V600E, KRAS and NRAS were detected

by PCR and direct sequencing. Sequencing was performed with the ABI BigDye v3.1 dye terminator sequencing chemistry and the ABI PRISM 3730×l capillary DNA analyzer at the UCSF Genomic Core Facility. The sequences were analyzed to determine mutation status using Mutation Surveyor v3.10 (SoftGenetics, State College, PA). RET/PTC1, RET/PTC3 and NTRK1 rearrangements were detected by nested PCR. The primer sets used to detect point mutations and rearrangements are listed in Table 1.

Data Analysis

The data are presented as proportion, number or mean ± standard deviation (SD). Time-trend analyses of demographic, clinical, pathologic, and somatic mutation status (BRAF, NRAS, KRAS, RET/PTC1 and RET/PTC3 rearrangements, NTRK1 rearrangement) were performed over three 5 year time periods; Group I (1991–1995), Group II (1996–2000), and Group III (2001–2005). Differences between time periods for categorical data were analyzed using χ^2 test and for continuous or nonparametric data using analysis of variance or Kruskal-Wallis test, respectively. All analyses were performed using Statview. A p value < 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the study cohort across the three 5-year time intervals are summarized in Table 2. There was no significant difference among the three time groups in terms of age at diagnosis, gender, ethnicity, primary tumor size, TNM cancer stage, or rate of extrathyroidal invasion in the entire study cohort. The majority of the patients in each group were Caucasian women with TNM Stage I. In patients for whom there was primary tumor sample available for molecular analysis (N=228), there was also no significant difference in age at diagnosis, gender, ethnicity, TNM cancer stage, or rate of extrathyroidal invasion among the three time groups. The primary tumor size, however, was significantly smaller in Group III as compared to Groups I and II (average tumors size of 1.8 cm vs. 2.4 and 2.6 cm, respectively) (p value < 0.001). Group III also had significantly more occult papillary thyroid cancer (≤ 1 cm) as compared to Groups I and II (30% vs. 7% and 5%, respectively) ($p < 0.001$) (Table 3).

Somatic mutations were detected in 164 tumor samples (72%) (Table 4). There was a significantly higher rate of somatic mutation in Group III (92%) as compared to Groups I and II (68% and 64%, respectively) ($p \leq 0.002$). The most common genetic change detected was BRAF V600E mutation, which was also significantly higher in Group III (88%) as compared to Groups I and II (51% and 43%, respectively) ($p<0.001$). There was no significant difference among the less frequent genetic changes by time period. The significantly higher rate of BRAF V600E mutation in Group III was independent of clinical and pathologic variables. The thyroglobulin gene copy level, by quantitative PCR, was not significantly different by time period.

DISCUSSION

In this study, we performed a time-trend analysis of demographic, clinical, pathologic, and tumor mutational status of patients with papillary thyroid cancer over a fifteen year time period. Our analysis shows that although demographic, clinical and pathologic factors did not change over this time period, the rate of somatic mutations, especially BRAF V600E, increased significantly.

Thyroid cancer is one of the most rapidly increasing cancer diagnoses worldwide. Several investigators have suggested based on cancer registry data that the increase in incidence may be due to diagnostic scrutiny. For example, Davies and Welch analyzed the Surveillance,

Epidemiology, and End Results (SEER) database from 1973 to 2002 and found that the increase in thyroid cancer was predominantly among small (≤ 2cm) papillary thyroid cancers (3). Kent and associates also found a similar increase in the number of papillary thyroid cancers ≤ 2 cm in size in Ontario, Canada (10). Therefore, it has been suggested that the increase in thyroid cancer incidence is a result of diagnostic scrutiny and detection of subclinical disease. In contrast, Chen and associates recently evaluated the Surveillance, Epidemiology, and End Results database between 1988 and 2005 and found an increase in incidence rates for all sizes of tumor (1). They concluded that the increased incidence across all tumor sizes could not solely be due to increased detection. In our study population, we observed no significant difference in demographic, clinical and pathologic factors over a 15 year period. In patients for whom there was tumor tissue available for molecular analysis, the average tumor size was however smaller in Group III as compared to Groups I and II, and the proportion of occult papillary thyroid cancer (<1 cm) was higher.

Family history of thyroid cancer, head and neck irradiation and ionized radiation exposure are well-established risk factors for thyroid cancer. Over the study time period, there has been one significant radiation population exposure as a result of the Chernobyl reactor meltdown, which has been documented to increase the rate of thyroid cancer in children from regions with significant exposure (11). However, there has been no significant change in these risk factors at a national or international level to explain the marked increase in thyroid cancer incidence seen across different regions and continents (5–8). Our patient population has a significant Russian patient population but ethnicity across the three different time periods was similar. In a recent analysis of county specific thyroid cancer registry data in the United States, higher incidence rates were observed in regions within 90 miles of nuclear power reactor plants (12). At this time it is unclear what the level of radiation exposure is in this situation and whether such level of radiation exposure would increase the risk of thyroid cancer. Higher ionized radiation exposure as a result of the Chernobyl meltdown have been associated with RET/PTC3 rearrangements in children with papillary thyroid cancer but not BRAF V600E mutations (11,13).

Our main objective was to evaluate the rate of molecular changes that have occurred over time. To our knowledge, this is the first study to evaluate mutational status in thyroid tumors over time. Interestingly, there was a striking difference in presence of not only any somatic mutation in Group III as compared to Group I and II, but specifically BRAF V600E mutation ($p<0.001$ for both). Group III had an overall 92% somatic mutation rate and an 88% BRAF V600E mutation rate, significantly higher than in the other two groups. This increase in somatic mutation rate was independent of clinical and pathologic variables which were similar across the three time periods for the entire cohort as well as for those only for which tumor sample was available, except for tumor size. This would suggest that a rise in BRAF mutation rate may account for the higher incidence of papillary thyroid cancer. It is unlikely that longer period of tumor sample storage or tumor sample quality affected the mutation rates over time as the quality of the nucleic acid extracted was similar across the three time periods, and the thyroglobulin gene copy level was similar across the three time periods. The precise reason for such an increase in BRAF V600E mutation over time is unclear but may reflect increased exposure to chemical carcinogenes or other environmental factors that may make this mutation more prevalent.

One of the limitations of our study is that it was performed in a cohort from a single institution and thus may not be generalizable. However, such an analysis of a cohort from a single institution allows for more detailed analysis of demographic and clinical characteristics in conjunction with the mutation analysis of tumor samples. At the very least our single institution study provides data for future studies to explore this finding as a reason for the increasing incidence of thyroid cancer, especially given both in vitro and in vivo

studies of activating BRAF mutation have demonstrated this oncogene is involved in the initiation and progression of papillary thyroid cancer (14,15). The presence of BRAF mutations in papillary thyroid cancer have also been associated with more aggressive disease (e.g. higher rates of extrathyroidal invasion and lymph node metastasis) and higher rate of disease recurrence, even in low risk papillary thyroid cancer cases (16,17). The higher rate of BRAF V600E mutation observed in Group III is not likely to be due to differences in disease aggressiveness as the extent of disease across all three groups were similar and the subset of tumors analyzed in Group III were also similar to the entire cohort of patients for that time period (Tables 2 and 3). Another possible weakness in our study is risk factors such as radiation exposure and family history could not be determined in the study population which thus may influence the mutation rate detected. However, radiation exposure is not likely to affect the BRAF V600E mutation rate. And while the association of radiation exposure and RET/PTC3 rearrangements is strong this mutation was observed at a relatively low rate and was not different over time (11). Lastly, we recognize that an increase in the rate of BRAF mutation during a time in which the incidence of thyroid cancer has doubled does not necessary demonstrate a cause and effect but provides data that should be explored in future studies to understand the factors associated with the increasing incidence of thyroid cancer.

In conclusion, we found higher rates of BRAF V600E mutation over a 15 year period. Our findings suggest a higher rate of somatic mutations in papillary thyroid cancer thus may contribute to the increasing incidence of papillary thyroid cancer. The reason for the increased rate of somatic mutations will need to be evaluated in future studies.

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Table 1

PCR primers for BRAF, KRAS and NRAS hotspot mutations, and RET/PTC1, RET/PTC3 and NTRK1 chromosomal rearrangements

Gene	Codons	Primer Sequences (5'–3'; a, forward; b, reverse)
KRAS	12/13	a 5'-GGCCTGCTGAAAATGACTGAA-3'
		b 5'-GGTCCTGCACCAGTAATATGC-3'
KRAS	61	a 5'-CAGGATTCCTACAGGAAGCAAGTAG-3'
		b 5'-CACAAAGAAAGCCCTCCCCA-3'
NRAS	12/13	a 5'-ATGACTGAGTACAAACTGGT-3'
		b 5'-CTCTATGGTGGGATCATATT-3'
NRAS	61	a 5'-TCTTACAGAAAACAAGTGGT-3'
		b 5'-AGCGGATAACAATTTCACACAGGC CAA AAA TTTAATCAGTGGA-3'
BRAF	600	a 5'-TGTAAAACGACGGCCAGTCATAATGCTTGCTCTGA TAG GA-3'
		b 5'-AGCGGATAACAATTTCACACAGGCCAA AAATTTAATCAGTGGA-3'
RET/PTC1		a 5'-GCT GGA GAC CTA CAA ACT GA-3'
		b 5'-GTT GCC TTG ACC ACT TTT C-3'
	Nested primer	a 5'-ACA AAC TGA AGT GCA AGG CA-3'
		b 5'-GCC TTG ACC ACT ACT TTT CCA AA-3'
RET/PTC3		a 5'-AAG CAA ACC TGC CAG TGG-3'
		b 5'-CTT TCA GCA TCT TCA CGG-3'
	Nested primer	a 5'-CCT GCC AGT GGT TAT CAA GC-3'
		b 5'-GGC CAC CGT GGT GTA CCC TG-3'
NTRK1		a 5'-TGAGCAGATTAGACTGATGG-3'
		b 5'-GGAAGAGGCAGGCAAAGAC-3'
	Nested primer	a 5'-GCTGCCGAAGAAAAGTACTC-3'
		b 5'-TTTCGTCCTTCTTCTCCACC-3'

Table 2

Demographic and Clinicopathologic Characteristics of study cohort over three time periods

	Group I (n=198) 1991–1995	Group II (n=202) 1996–2000	Group III (n=228) 2001–2005	P-value
Average age at diagnosis (years) ± SD	41.3 ± 15.0	44.0 ± 17.6	42.7 ± 15.1	≥0.27
Gender				
Women (%)	77%	76%	78%	≥0.81
Ethnicity				
Caucasian	80%	78%	77%	≥0.07
Asian	16%	17%	18%	
Black	4%	2%	2%	
Other	0%	3%	3%	
Largest Tumor Diameter (cm)	1.6 ± 1.2	1.6 ± 1.3	1.7 ± 1.7	≥0.95
TNM Stage				≥0.54
I	136	132	167	
II	26	18	14	
III	29	43	39	
IV	7	5	8	
Extrathyroidal invasion (%)	18	17	15	≥0.62
Lymph node metastasis (%)	29	29	32	≥0.77

Table 3

Demographic and Clinical Characteristics of 228 cases with tumor sample available for mutation analysis over three time intervals

	Group I (n=74) 1991–1995	**Group II (n=103) 1996–2000**	**Group III (n=51) 2001–2005**	**P-value**
Average age at diagnosis (years) ± SD	46.4 ± 18.8	43.5 ± 18.1	48.7 ± 16.4	≥0.33
Gender				
Female (%)	74%	69%	81%	≥0.32
Male (%)	26%	31%	19%	
Ethnicity				
Caucasian	76%	74%	76%	≥0.80
Asian	24%	24%	22%	
Black	0	2%	2%	
Other				
Largest Tumor Diameter (cm) Mean ± standard error of the mean	2.36 ± 0.18	2.59 ± 0.16	1.82 ± 0.28	≤ 0.001*
TNM Stage				≥0.54
I	50	68	36	
II	11	14	3	
III	13	21	9	
IV	0	1	3	
Extrathyroidal invasion (%)	17	16	15	≥0.66
Lymph node metastasis (%)	28	27	30	≥0.46

* For comparison of Group I and or II to Group III.

Table 4

Molecular testing results for 228 cases of conventional papillary thyroid cancer

	Group I (n=74) 1991–1995	Group II (n=103) 1996–2000	Group III (n=51) 2001–2005	P-value
Somatic mutation	68% (51/74)	64% (66/103)	92% (47/51)	≤ 0.002*
BRAF mutation	51% (38/74)	43% (44/103)	88% (45/51)	<0.001*
RET/PTC1	0%	3% (3/103)	0%	0.15
RET/PTC3	14% (10/74)	16% (16/103)	4% (2/51)	0.18
NRAS	1% (1/74)	1% (1/103)	0%	0.44
KRAS	3% (2/74)	2% (2/103)	0%	0.52

* For comparison of Group I and or II to Group III.