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INCREASED INCIDENCE OF WELL-DIFFERENTIATED THYROID CANCER ASSOCIATED WITH HASHIMOTO'S THYROIDITIS AND THE ROLE OF THE PI3K/AKT PATHWAY

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Abstract

BACKGROUND—The link between inflammation and cancer is well-established; however, the link between Hashimoto's thyroiditis (HT) and thyroid cancer remains controversial. The purpose of our study was threefold: 1) to determine the incidence of patients with thyroid cancer and associated HT at our institution, 2) to correlate our patient population demographics with the Surveillance, Epidemiology and End Results (SEER) database, and 3) to assess the expression of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in patients with HT.

STUDY DESIGN—Demographic and histological data was collected from patients undergoing thyroid resection at the University of Texas Medical Branch (UTMB) from 1987 to 2002 and compared to the SEER database. Immunohistochemistry for phosphorylated Akt (a marker of PI3K activity), Akt isoforms and PTEN (an inhibitor of PI3K) was performed on paraffin-embedded blocks of resected thyroid tissue.

RESULTS—Our patient population demographics and thyroid cancer incidence by histological type were similar to patients in the SEER database. Ninety-eight (37.7%) resected specimens had pathological changes consistent with HT; 43 (43.8%) had an associated well-differentiated thyroid cancer. Increased p-Akt, Akt1 and Akt2 expression was noted in regions of HT and thyroid cancer compared to regions of normal surrounding thyroid tissue.

CONCLUSIONS—Patients with HT were 3 times more likely to have thyroid cancer suggesting a strong link between chronic inflammation and cancer development. PI3K/Akt expression was increased in both HT and well-differentiated thyroid cancer suggesting a possible molecular mechanism for thyroid carcinogenesis.

Keywords

Well-differentiated thyroid cancer; Hashimoto's thyroiditis; PI3K; Akt 1; Akt 2; PTEN; SEER database

INTRODUCTION

Thyroid cancer is the most common endocrine malignancy in the United States with approximately 25,000 new cases in 2005 alone¹. Papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) are classified as well-differentiated thyroid cancers accounting for approximately 95% of all thyroid cancers and generally have a favorable prognosis². The transformation of normal (ie, benign) tissue to a cancer is a multistep and multifactorial process in which numerous factors have been implicated including environmental exposure, loss of tumor suppressor genes and chronic inflammation³.

Chronic inflammation, leading to neoplastic transformation, is a well-established clinical phenomenon⁴. Inflammation elicits an immune response with activation of chemokines/ cytokines and growth factors leading to damage of surrounding stromal cells⁵. This cycle of repeated cellular damage and subsequent healing alters stromal elements contributing to genetic alterations, inappropriate cell proliferation and subsequent neoplastic transformation³. Hashimoto's thyroiditis (HT) is an autoimmune inflammatory disease characterized by widespread lymphocyte infiltration, fibrosis and parenchymal atrophy⁶. HT affects approximately 5% of the population, is usually diagnosed in the 4th – 6th decade of life and is approximately 15 times more common in women⁶. Historically, the presence of HT was thought to increase the risk of developing thyroid lymphoma. Dailey et al⁷ in 1955, however, reported an increased association between HT and PTC, but not lymphoma. Since this initial report, the causal association of the two diseases remains controversial with various authors reporting no association between HT and PTC while others describe a variable frequency as high as 38%^{6, 8–12}. HT and PTC share many morphological and molecular features, but the clinical implications and importance of this correlation is unknown.

Alterations in cell signaling pathways with subsequent loss of cell cycle control mechanisms have been implicated in neoplastic transformation¹³. Phosphatidylinositol 3-kinase (PI3K), a ubiquitous lipid kinase that is activated by a wide variety of extracellular stimuli, plays a critical role in the balance between cell survival and apoptosis^{14, 15}. Additionally, PI3K is important in the inflammatory response by activating chemokine receptors and promoting leukocyte migration¹⁶. Increased PI3K activation has been identified in a number of cancers including colorectal, ovarian and thyroid^{17–20}. PI3K activation, in turn, phosphorylates (ie, activates) Akt which acts on downstream proteins to suppress proapoptotic signals further contributing to tumorigenesis¹⁴. Three isoforms of Akt exist in humans, each with a specific role in PI3K-mediated cellular responses²¹. Ringel et al¹⁸ reported increased Akt1 and Akt2 expression in FTC, but they did not observe this induction in PTC. The tumor suppressor protein PTEN is a natural inhibitor of PI3K^{22, 23}. Mutations of PTEN are associated with Cowden's disease in which more than 50% of patients develop follicular thyroid neoplasia²⁴. Decreased PTEN expression has also been described in cases of sporadic FTC^{25, 26}. The role of PI3K/Akt and PTEN has, to our knowledge, not been investigated in patients with HT, PTC or HT with associated well-differentiated thyroid cancer.

Anecdotally, we have noticed an increased incidence of HT associated with thyroid cancer at our institution. Therefore, the purpose of our current study was threefold: 1) to determine the incidence of patients with thyroid cancer and associated HT at our institution, 2) to correlate our patient population demographics with the larger National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database, and 3) to assess the expression of the PI3K/Akt pathway components in patients with HT, well-differentiated thyroid cancer (PTC and FTC) and HT with concurrent thyroid cancer. Here, we demonstrate an increased incidence of well-differentiated thyroid cancers associated with HT. Additionally, we found increased expression of phosphorylated Akt (p-Akt), Akt1, and Akt2 in HT alone, HT with well-differentiated cancer and thyroid cancer alone; decreased PTEN expression was noted in

thyroid cancer. Increased Akt1 and Akt2 expression and a loss of PTEN function likely contributes to tumorigenesis demonstrating that the PI3K/Akt pathway plays crucial role in HT and HT associated with well-differentiated thyroid cancer.

MATERIALS AND METHODS

Study design

The histopathology and clinical course of patients undergoing thyroid resection from January 1, 1987 to December 31, 2002 at The University of Texas Medical Branch (UTMB) were retrospectively analyzed. UTMB Institutional Review Board approval was obtained for the collection of patient data, tissue acquisition and subsequent use. A comprehensive search of the medical records was first performed using Common Procedure Terminology (CPT) codes for “thyroid resection” (excluding closed biopsy), “thyroid cancer” (all subtypes), “Hashimoto’s thyroiditis” and “thyroiditis.” Patients undergoing closed or open biopsy without further formal resection, thyroid resection for other diseases (eg, laryngectomy, parathyroid resection), patients with non-specific thyroiditis or who had previously undergone partial thyroid resection were excluded. Histopathology reports were then obtained for all remaining patients during the specified time period. Patients with a pathologically confirmed diagnosis of thyroid cancer (papillary, follicular, medullary, anaplastic or lymphoma) and/or Hashimoto’s thyroiditis (defined by the presence of diffuse lymphocytic and plasma cell infiltrates, oxyphilic cells and lymphoid follicles with reactive germinal centers) were then entered into the UTMB Thyroid Database. Patients with HT immediately surrounding the tumor (ie, with no other lobe involvement) were considered as having thyroid cancer alone since the surrounding inflammation may represent a desmoplastic reaction to the tumor. Demographic data (age, sex, race), TNM stage, tumor size (greatest dimension), number of lymph nodes resected, number of positive lymph nodes, presence or absence of HT, presence of distant metastasis, and presence of extrathyroid extension were collected for all cases. For tissue analysis, paraffin-embedded blocks of resected thyroid tissue (with surrounding normal thyroid tissue) were obtained from 34 randomly selected patients with no thyroid disease, a diagnosis of HT alone, thyroid cancer alone, or HT with associated thyroid cancer. Blocks were sectioned for immunohistochemistry.

SEER database

The National Cancer Institute’s SEER-9 program was utilized to collect national data on thyroid cancer incidence, patient age and sex distribution, tumor histology, TNM stage, lymph node status, and tumor size for the years 1987 – 2002. SEER data is compiled from population-based cancer data from 9 cancer registries in geographically distinct areas of the United States (5 states: Connecticut, Hawaii, Iowa, New Mexico and Utah; 4 cities: Atlanta, Detroit, San Francisco and Seattle)¹. Population data for further statistical analysis was obtained from the 2000 United States Census Bureau (www.census.gov).

Immunohistochemistry

Paraffin-embedded thyroid blocks were sectioned (5 µm) and deparaffinized in xylene and rehydrated in descending ethanol series. Immunostaining was performed using DAKO EnVision Kit (Dako Corp., Carpinteria, CA) as we have previously described¹⁹. Briefly, sections were incubated overnight at 4°C with monoclonal antibodies diluted 1:100 in 0.05M Tris-HCL + 1% BSA against anti-Akt1, anti-Akt2, anti-pAkt (Ser473), and anti-PTEN (Cell Signaling, Beverly, MA). After 3 washes with TBST, the sections were incubated for 30 min with secondary antibody labeled with peroxidase, then washed 3 times with TBST. Lastly, peroxidase substrate DAB was added for staining. All sections were counterstained with hematoxylin and observed by light microscopy. For negative controls, primary antibody was

omitted from the above protocol. All specimens were reviewed by a pathologist in a blinded fashion.

Statistical analysis

Association between well-differentiated thyroid cancer and HT was assessed using the Pearson chi-square test. Comparisons of UTMB data with SEER data were tested using the Pearson chi-square test for histological type, gender, race, and tumor stage and using the median test for age, tumor size, and number of positive lymph nodes. Association between type of thyroid cancer and data set (UTMB or SEER) controlling for gender or race was assessed using the Cochran-Mantel-Haenszel test. Associations between HT (present or absent) and prognostic variables in patients with well-differentiated thyroid cancer (PTC or FTC) were assessed using the Pearson chi-square test for gender, tumor stage, positive lymph node (positive or negative) and extra-thyroid extension (present or absent) and using the median test for age tumor size. All statistical computations were carried out using statistical software, SAS®, Release 9.1²⁷.

RESULTS

UTMB patient demographics

Between January 1, 1987, and December 31, 2002, 812 patients underwent thyroid resection at UTMB. Of these, 260 patients were identified as having primary thyroid cancer, HT, or HT associated with thyroid cancer (162, 52, and 46 patients, respectively). The remaining 552 patients had diagnoses other than thyroid cancer or HT and were excluded from the study. Two patients with primary thyroid lymphoma, one patient with insular thyroid carcinoma (an unusual poorly differentiated cancer), and one patient with Hürthle cell carcinoma without definitive evidence of follicular cell involvement were excluded from further analysis. There was a total of 161 women (78.9%) and 43 men (21.1%) with a median age of 40 (range 7–79). Caucasians represented 49% of all patients, followed by Hispanic (33.2%), African American (13.9%), and Asian/Pacific Islander (3.5%). Total thyroidectomy was performed in 55.8% of resections and 99 patients (38.4%) underwent lymph node dissection with an average of 7.5 nodes removed. Primary thyroid malignancy was identified in 204 patients (25.1%); PTC was the most common histological type identified (87.6%), followed by FTC (10.4%), medullary (0.5%) and anaplastic (1.5%). Median tumor size was 2.0 cm (range 0.09 – 9.0 cm) and 83.7% of patients had tumor confined to the thyroid. Lymph node metastases were present in 70 patients (34%), 4 patients (2.0%) had evidence of distant metastases, and 137 (68.8%) had Stage I disease (Stage II: 10.6%; III: 14.1%; IV: 6.5%).

Comparison of UTMB patients with the SEER database

Demographics for patients with thyroid cancer resected at UTMB and those recorded in the SEER database are presented in Table 1. A search of the SEER database identified 22,647 patients with PTC, FTC and poorly differentiated (medullary and anaplastic) thyroid cancer during the study period. The two populations had a similar ratio of female patients, median tumor size and histological type. The median age was slightly higher in SEER patients (44 vs. 40; $p=0.004$). Racial and clinical stage distributions were also noted to be statistically different ($p<0.0001$ for both factors) between the two populations. UTMB had fewer Caucasian and Asian patients and a higher number of African-American and Hispanic patients compared to the SEER database consistent with the demographic make-up of the state of Texas. While the histological type was similar, the stage distributions differed between the 2 populations. The majority of SEER patients were designated Stage I (83.7%) followed by Stage II (7.7%), whereas 68.8% of UTMB patients were Stage I followed by Stage III (14.1%).

To further examine these populations, patients were stratified by gender and histological type of cancers (Table 2). The frequency of histological type by gender was similar between the

UTMB and SEER patients. There were no statistical differences when these two groups were compared for an association between thyroid cancer and the populations controlling for gender ($p=0.19$). UTMB and SEER patients were then stratified by race and histological type (Table 3). Again, the frequency of histological type by race was similar between the two populations with no association between thyroid cancer when controlling for race ($p=0.21$).

Comparison of HT and HT associated with thyroid cancer

Table 4 lists univariate prognostic factors for UTMB patients with thyroid cancer stratified by the presence or absence of HT. Overall, 98 patients were identified as having pathological changes consistent with HT (37.7%), 86 (88%) were with a median age of 44 (range 14–73). Of these cases, 46 patients (47%) had associated thyroid malignancy (all histological types). PTC was the most common histological type (74%), followed by FTC (19.6%), lymphoma (4.3%), and anaplastic thyroid cancer (2.2%). Patients with HT and associated cancer were similar to patients with thyroid cancer alone in regards to median age (41 to 39; $p=0.64$), racial distribution ($p=0.61$) and median tumor size (2.15 to 1.90 cm; $p=0.29$). A significantly higher percentage of women had HT and associated thyroid cancer when compared to thyroid cancer alone (90.7 vs. 75.5, respectively; $p=0.03$). While the overall racial distribution was similar ($p=0.61$), we observed that Hispanic patients had a lower incidence of HT and associated cancer compared to cancer alone (25.6% vs. 35.5%). Meanwhile, Caucasians and African-American had a slightly higher incidence of HT and associated cancer and thyroid cancer alone (53.5% vs. 47.7%, 18.6% vs. 12.3%, respectively).

The presence of positive lymph nodes and extrathyroid tumor extension were also assessed. Six patients (14%) with HT and associated thyroid cancer had extrathyroid extension compared to 38 (24.5%) patients with thyroid cancer alone; however, this was not statistically significant ($p=0.14$). The percentage of patients with positive lymph nodes between the groups was also not statistically significant (27.9 vs. 35.1; $p=0.38$). There was no statistical difference in clinical stage between patients with HT and cancer and cancer alone. Patients with HT alone had a higher median age when compared to patients with HT and cancer (48 vs. 41, respectively). Our patients with HT and associated thyroid cancer were similar to UTMB thyroid cancer patients with respect to demographics and prognostic variables; however, there was a significantly higher percentage of women with HT and associated cancer. Overall, UTMB patients with HT were approximately 3 times more likely to have well-differentiated thyroid cancer when compared to patients with no HT (odds ratio 2.96; 95% CI: 1.9 – 4.6).

Expression of the PI3K pathway components in HT, papillary carcinoma, and corresponding normal thyroid

Histologically normal samples from patients undergoing thyroid resection for laryngeal carcinoma (control; $n=10$), HT ($n=8$), PTC ($n=8$), or HT associated with well-differentiated cancer (PTC; $n=8$) were analyzed for expression patterns of the PI3K/Akt pathway components p-Akt, Akt1, Akt2, and the tumor suppressor PTEN, the natural PI3K inhibitor. Stained specimens were then reviewed by a pathologist and graded according to expression levels. These results are summarized in Table 5. Samples were compared to H&E staining of normal, HT, HT-associated thyroid cancer and thyroid cancer alone (data not shown).

Representative tissue staining patterns from selected patients are shown in Figures 1 – 4. Similar sections of normal thyroid, thyroid with HT, HT with associated thyroid cancer and well-differentiated thyroid cancer alone were chosen to better illustrate changes in the expression of various proteins. These samples were representative of all specimens analyzed. Phosphorylated Akt was not expressed in the control tissue (Fig. 1A). Histologically, HT is characterized by diffuse lymphocytic infiltration, parenchymal atrophy, and fibrosis, leading to a derangement in follicular cells referred to as Hürthle cell change, with an increase in cell

size, acidophilic staining, shrinking of the follicular spaces, and sparse colloid deposition. In contrast to control thyroid tissues, HT specimens showed high levels of p-Akt expression within infiltrating lymphocytes and Hürthle cell changes with both a cytoplasmic and nuclear distribution (Fig. 1B). There was no expression in normal follicular cells. Phosphorylated Akt expression was also found to be high in HT associated thyroid cancer within papillary carcinoma cells, within areas of Hürthle cell changes and within infiltrating lymphocytes (Fig. 1C); residual normal follicles had no expression. Thyroid cancer alone (PTC) was noted to have p-Akt expression to a lesser extent again with a cytoplasmic and nuclear distribution (Fig. 1D).

We next analyzed specimens for Akt1 and Akt2 isoform expression. No Akt1 or Akt2 expression was noted in control thyroid tissues (Figs. 2A and 3A, respectively). Similar to p-Akt, Akt1 and Akt2 expression in HT alone (Figs. 2B and 3B, respectively) and HT associated thyroid cancer specimens (Figs. 2C and 3C, respectively) was high in areas of infiltrating lymphocytes and Hürthle cell changes with a cytoplasmic and nuclear distribution. There was no expression of either Akt isoforms in normal follicular cells. There was significant expression of Akt1 and Akt2 within papillary carcinoma cells with a distinct absence of staining within normal follicular cells (Figs. 2D and 3D, respectively).

Finally, we analyzed specimens for the tumor suppressor protein PTEN (Fig. 4). In contrast to p-Akt, Akt1 and Akt2, PTEN expression was expressed in normal thyroid specimens (Fig. 4A). There was positive PTEN cytoplasmic and nuclear expression in the follicular cells lining the thyroid follicles. PTEN expression in HT specimens was similar to p-Akt, Akt1 and Akt2 expression with high expression within infiltrating lymphocytes and areas of Hürthle cell changes (Fig. 4B). Similar to control tissues, the distribution was both cytoplasmic and nuclear. HT-associated thyroid cancer specimens had PTEN expression in infiltrating lymphocytes and areas of Hürthle cell changes; there was little, if any, PTEN expression in papillary carcinoma cells (Fig. 4C). Papillary carcinoma specimens had no PTEN expression (Fig. 4D).

In summary, the PI3K pathway components p-Akt, Akt1, and Akt2 were highly expressed in HT, HT-associated thyroid cancer and thyroid cancer alone with no expression noted in normal follicular cells. Conversely, the tumor suppressor PTEN was expressed in normal thyroid, with slightly increased expression in infiltrating lymphocytes and within areas of Hürthle cell change, but there was little, if any, expression of PTEN noted in papillary carcinoma cells (Table 5). These results, demonstrating increased PI3K activation and Akt isoform expression in areas of inflammation and cancer, suggest a possible common molecular mechanism between these two processes.

DISCUSSION

Since the initial description by Marjolin in 1828, chronic inflammation has been increasingly recognized as playing a major role in the development of cancers from multiple sites¹³. However, the association between HT and well-differentiated thyroid cancer remains controversial. In our current study, we demonstrate a 43.8% incidence of HT and associated well-differentiated thyroid cancer with an overall odds ratio of 2.96, which is higher than previously reported in other studies^{6, 8–12, 28}. In a population-based study of 200 patients with HT, Crile et al⁸ found only 1 case of PTC during a follow-up of more than 1000 patient-years. Holm et al⁹, in another large population-based study, showed only an increased association between HT and lymphoma. Conversely, Ott et al¹⁰ reported a 38% incidence of thyroid cancer associated with HT in 161 patients with thyroid cancer. Singh et al¹² reviewed 388 patients and found HT associated with PTC in 15% with an overall odds ratio of 1.89. Most recently, Cipolla et al⁶ reviewed 89 patients and showed a 27% incidence of HT associated thyroid cancer. The large variation in incidence between studies likely reflects

differences in the pathological definitions and interpretation of HT. Non-specific lymphocytic thyroiditis, occurring immediately adjacent to a tumor, may represent peri-neoplastic inflammation and not true HT. Furthermore, improvements in diagnostic capabilities have likely led to an increased identification of PTC²⁹.

Arif et al³⁰ have concluded that PTC and HT share many morphological and immunohistochemical patterns raising the question of whether HT is, in fact, a microscopic counterpart of PTC. To avoid over-diagnosis in our study, patients with an uncertain diagnosis of HT were excluded from analysis. Furthermore patients with HT or inflammation immediately adjacent to thyroid tumors were classified as having thyroid cancer alone. One potential limitation of this study is that we were unable to assess the length of time from the diagnosis of HT to the development of cancer. Additionally, our data is based on patients who underwent thyroid resection which will undoubtedly introduce a selection bias into our study population. Many patients with HT are not treated surgically and likely do not develop thyroid cancer making the true incidence difficult to calculate.

Loh et al³¹ recently examined the association between HT and thyroid cancer and concluded that patients with PTC, in the presence of HT, had a more favorable clinical outcome in regards to recurrence and mortality. We noted no differences between our populations with regards to extrathyroid tumor extension or positive lymph node status. Due to our study design, data on thyroid cancer recurrence or mortality was not available and therefore not analyzed. Overall, based on this and similar studies, we conclude that the presence of HT is associated with an increased incidence of thyroid cancer. Patients with HT should therefore be observed closely for neoplastic changes. Based on the findings of our current study, we would not advocate a more aggressive treatment for well-differentiated thyroid cancer that is found in association with HT.

The PI3K/Akt pathway plays a critical role in the balance between cell survival and apoptosis and the inflammatory response by activating chemokine receptors and promoting leukocyte migration^{14, 16}. Increased PI3K activity has been identified in colorectal, ovarian and thyroid cancers^{17–20}. To our knowledge, the role of the PI3K/Akt pathway in HT and HT associated cancers has not been previously examined. Phosphorylated Akt was noted in infiltrating lymphocytes and areas of Hürthle cell changes in the HT component of cancerous and non-cancerous tissues. Patients with thyroid cancer alone had increased expression of p-Akt in areas of pathologically confirmed PTC, but to a lesser extent than in HT and HT-associated cancers. The possible role of the PI3K/Akt pathway in thyroid cancers has been described in a few reports. Ringel et al¹⁸ showed increased Akt phosphorylation and expression in thyroid cancer cell lines. Total and phosphorylated Akt isoforms were increased in FTC, but not PTC. Vasko et al²⁰ noted that p-Akt was increased in regions of histologically defined capsular invasion in 26 of 26 PTC's and 10 of 10 FTC's. Finally, Miyakawa et al¹⁷ noted significantly increased levels of p-Akt and p70S6K (another downstream target of PI3K) in thyroid tumors compared to normal thyroid tissue. The presence of activated Akt in regions of chronic inflammation and thyroid cancer, as demonstrated here, suggests that the PI3K/Akt pathway plays a common role in neoplastic transformation in the presence of HT.

We have further examined the expression of specific Akt isoforms and noted increased levels of Akt1 and Akt2 expression in HT and in cancers with no expression in control tissues. Increased Akt has been identified in various cancers including prostate, breast, ovarian, pancreas, colon and thyroid^{32, 33}. Recent reports have focused on the specific roles for Akt isoforms. Akt1 is thought to be important for PI3K-mediated cell proliferation and may act to suppress cancer invasion. Yoeli-Lerner et al³⁴ reported increased cell proliferation and decreased lung metastases with Akt1 activation in a murine model. However, this remains controversial and the focus of ongoing research. Akt2 expression appears to be important for

the invasion of various cancer cell types. Arboleda et al³⁵ reported increased adhesion and invasion in 8 separate human breast and ovarian cancer cell lines in which Akt2 was overexpressed. Recently, we demonstrated increased Akt2 expression in advanced stage colorectal cancers. In this study, Rychahou et al¹⁹ demonstrated strong Akt2 expression in the stromal epithelium and inflammatory cells. The similar distribution patterns of Akt isoforms in HT and thyroid cancer lends further evidence that PI3K/Akt is a common signaling pathway for these conditions. PI3K/Akt activation in response to chronic inflammation likely leads to suppression of pro-apoptotic mechanisms and promotion of cell proliferation with eventual neoplastic transformation.

The tumor suppressor protein PTEN plays an important role in regulating the PI3K pathway with loss of PTEN function associated with a variety of human cancers including thyroid^{23, 36}. Patients with inherited mutations in the PTEN gene have a higher incidence of FTC and loss of PTEN expression has been reported in 15 – 30% of sporadic thyroid cancers^{25, 26, 37}. We noted PTEN expression in regions of HT (±cancer); expression was absent in thyroid cancers. The loss of PTEN expression may contribute to tumorigenesis in patients with HT-associated thyroid cancers.

In conclusion, we have shown a significantly higher incidence of HT-associated thyroid cancer in patients with HT undergoing resection than has previously been reported. Patients with HT were 3 times more likely to demonstrate an associated well-differentiated thyroid cancer compared to patient without HT suggesting a link between chronic inflammation and cancer development. A higher percentage of women had HT and associated thyroid cancer when compared to the population with thyroid cancer without HT. Increased Akt1 and Akt2 expression and a loss of PTEN function likely contributed to tumorigenesis demonstrating that the PI3K/Akt pathway plays an important role in these two closely related diseases.

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ABBREVIATIONS

HT	Hashimoto's thyroiditis
PTC	papillary thyroid cancer
FTC	follicular thyroid cancer
PI3K	phosphatidylinositol 3-kinase
SEER	Surveillance Epidemiology and End-Results
UTMB	The University of Texas Medical Branch

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Figure 1. Immunohistochemical analysis of representative thyroid tissues stained for p-Akt expression

(A, A') Normal thyroid showing absent p-Akt expression in normal follicular cells lining a thyroid follicle (arrow head). (B, B') Hashimoto's thyroiditis showing p-Akt expression in areas of Hürthle cell changes (white arrow) compared with normal follicles (arrowhead) (black arrow indicates large germinal center). (C, C') Hashimoto's thyroiditis with papillary carcinoma. The black arrow indicates Hürthle cell changes in a region of Hashimoto's thyroiditis with increased p-Akt expression. p-Akt expression is also increased in regions of papillary cancer (arrow head; white arrow points to a normal follicle). (D, D') papillary carcinoma showing increased p-Akt expression (arrow head signifies papillary carcinoma; the white arrow indicates a normal residual follicle). (Panels A – D = 100x; panels A' - D' = 600x)

Figure 2. Immunohistochemical analysis of representative thyroid tissues stained for Akt1 expression

(A, A') Absent Akt1 expression in normal follicular cells lining a thyroid follicle (arrow head). (B, B') Hashimoto's thyroiditis showing increased Akt1 expression in areas of Hürthle cell changes (white arrow) compared with normal follicles (arrowhead). The black arrow indicates large germinal center which also demonstrates increased Akt1 expression. (C) Hashimoto's thyroiditis with papillary carcinoma showing increased Akt1 expression lining the entire follicle affected by papillary cancer. The black arrow indicates Hürthle cell changes in a region of Hashimoto's thyroiditis with increased Akt1 expression. (C') Strong Akt1 expression is noted in regions of papillary cancer on higher magnification (arrow head; white arrow points to a normal follicle). (D, D') papillary carcinoma with strong Akt1 expression (arrow head signifies papillary carcinoma). (Panels A – D = 100x; panels A' - D' = 600x)

Figure 3. Immunohistochemical analysis of representative thyroid tissues stained for Akt2 expression

(A, A') Normal thyroid showing absent Akt2 expression in normal follicular cells lining a thyroid follicle (arrow head). (B, B') Hashimoto's thyroiditis with multiple large germinal centers (black arrows) showing increased Akt2 expression. The arrow head in the inset indicates a normal follicle, and the white arrow signifies Hürthle cell change with strong Akt2 expression. (C, C') Hashimoto's thyroiditis with papillary carcinoma (the black arrow indicates Hürthle cell changes with Akt2 expression, the arrow head points to a normal follicle, and the white arrow signifies papillary carcinoma, again, with strong Akt2 expression). (D, D') multiple regions of papillary carcinoma with increased Akt2 expression (the arrow head signifies papillary carcinoma). (Panels A – D = 100x; panels A' - D' = 600x)

Figure 4. Immunohistochemical analysis of representative thyroid tissues stained for PTEN
(A, A') PTEN expression is noted in normal thyroid tissue (arrow head points to follicular cells lining a normal thyroid follicle). **(B, B')** Hashimoto's thyroiditis with PTEN expression noted in areas of inflammation and Hürthle cell changes (the arrow head in the inset indicates a normal follicle, and the white arrow signifies Hürthle cell changes). The black arrow indicates a large germinal center **(C, C')** Hashimoto's thyroiditis with papillary carcinoma. The black arrow indicates a region of Hürthle cell change with PTEN expression noted. The arrow head points to a normal follicle with PTEN expression; the white arrow signifies papillary carcinoma with absent PTEN expression. **(D, D')** papillary carcinoma showing absent PTEN expression. The white arrow indicates a normal residual follicle; the arrow head signifies papillary carcinoma with absent PTEN expression. (Panels A – D = 100x; panels A' - D' = 600x)

Table 1

Comparison of UTMB patient demographics with SEER Database.

	UTMB	SEER Database	p-Value
Number of Cases of Thyroid Cancer	204	22647	
Age; median (range)	40 (7–79)	44 (4–99)	<i>0.004</i>
Sex: Female/Male; n (%)	161/43 (78.9/21.1)	17213/5434 (76/24)	<i>0.37</i>
Race (%)			<i><0.0001</i>
Caucasian	49.0	75.5	
African-American	13.9	5.3	
Hispanic	33.2	7.3	
Asian	3.5	10.2	
Tumor Size (cm); median (range)	2.0 (0.1–9)	1.8 (0–20)	<i>0.13</i>
TNM Stage; n (%)			<i><0.0001</i>
I	137 (68.8)	13807 (83.7)	
II	21 (10.6)	1271 (7.7)	
III	28 (14.1)	830 (5.0)	
IV	13 (6.5)	598 (3.6)	
Histological Type (%)			<i>0.18</i>
PTC	87.6	83.7	
FTC	10.4	12.7	
MTC	0.5	2.6	
ATC	1.5	1.1	

Table 2
Comparison of sex & frequency of thyroid cancer for UTM and SEER patients.

	PTC N (%)	FTC N (%)	MTC N (%)	ATC N (%)	Total Number
Female					
UTM	140 (88.1)	16 (10.1)	0 (0)	3 (1.9)	159
SEER	14652 (85.1)	2062 (12.0)	349 (2.0)	150 (0.9)	17213
Male					
UTM	37 (86.1)	5 (11.6)	1 (2.3)	0 (0)	43
SEER	4299 (79.1)	806 (14.8)	235 (4.3)	94 (1.7)	5434

Table 3
Comparison of race & frequency of thyroid cancer in UTMB and SEER patients.

		PTC N (%)	FTC N (%)	MTC N (%)	ATCN (%)	Total Number
Caucasian	UTMB	85 (85.9)	12 (12.1)	0 (0)	2 (2.0)	99
	SEER	14295 (83.6)	2152 (12.6)	467 (2.7)	190 (1.1)	17104
African-Am.	UTMB	22 (78.6)	5 (17.9)	1 (3.6)	0 (0)	28
	SEER	901 (74.8)	247 (20.5)	38 (3.2)	19 (1.6)	1205
Asian/Pac Isl	UTMB	6 (85.7)	1 (14.3)	0 (0)	0 (0)	7
	SEER	1992 (86.4)	252 (10.9)	43 (1.9)	20 (0.9)	2307
Hispanic	UTMB	63 (94.0)	3 (4.5)	0 (0)	1 (1.5)	67
	SEER	1453 (87.6)	171 (10.3)	23 (1.4)	12 (0.7)	1659

Table 4

Univariate prognostic variables in UTMB patients with well-differentiated thyroid cancer (PTC/FTC) stratified by the presence of HT.

	HT Present N = 43	HT absent N = 155	p-Value
Age; median (range)	41 (19–66)	39 (7–79)	<i>0.64</i>
Female gender; n, (%)	39 (90.7)	117 (75.5)	<i>0.03</i>
Tumor size (cm); median (range)	2.2 (0.1–5)	1.9 (0.1–8.5)	<i>0.29</i>
% of patients with positive lymph nodes; n, (%)	12 (27.9)	54 (35.1)	<i>0.38</i>
TNM Stage; n, (%)			<i>0.84</i>
I	28 (65.1)	109 (71.7)	
II	5 (11.6)	16 (10.5)	
III	7 (16.3)	20 (13.2)	
IV	3 (7.0)	7 (4.6)	
Extra-thyroid tumor extension; n, (%)	6 (14.0)	38 (24.5)	<i>0.14</i>
Race; n, (%)			<i>0.61</i>
Caucasian	23 (53.5)	74 (47.7)	
African- American	8 (18.6)	19 (12.3)	
Hispanic	11 (25.6)	55 (35.5)	
Asian	1 (2.3)	6 (3.9)	
Other	0 (0)	1 (0.6)	

Table 5

Summary of IHC staining results.

	Akt 1	Akt 2	p-Akt	PTEN
Normal	–	–	–	+
HT alone	++	++	++	+
Cancer alone	+	++	+	–
HT + Cancer	++	++	++	+/-

[(-) = absent staining; (+) = positive staining; (++) = strong positive staining; (+/-) = heterogeneous positive/absent staining]