# Clinical, Sonographic, and Pathological Characteristics of RAS-Positive Versus BRAF-Positive Thyroid Carcinoma

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**Context:** Mutations in the BRAF and RAS oncogenes are responsible for most well-differentiated thyroid cancer. Yet, our clinical understanding of how BRAF-positive and RAS-positive thyroid cancers differ is incomplete.

**Objective:** We correlated clinical, radiographic, and pathological findings from patients with thyroid cancer harboring a BRAF or RAS mutation.

**Design:** Prospective cohort study.

**Setting:** Academic, tertiary care hospital.

**Patients:** A total of 101 consecutive patients with well-differentiated thyroid cancer.

**Main Outcome Measure:** We compared the clinical, sonographic, and pathological characteristics of patients with BRAF-positive cancer to those with RAS-positive cancer.

**Results:** Of 101 patients harboring these mutations, 71 were BRAF-positive, whereas 30 were RAS-positive. Upon sonographic evaluation, RAS-positive nodules were significantly larger ($P = .04$), although BRAF-positive nodules were more likely to harbor concerning sonographic characteristics (hypoechogenicity [$P < .001$]; irregular margins [$P = .04$]). Cytologically, 70% of BRAF-positive nodules were classified positive for PTC, whereas 87% of RAS-positive nodules were indeterminate ($P < .001$). Histologically, 96% of RAS-positive PTC malignancies were follicular variants of PTC, whereas 70% of BRAF-positive malignancies were classical variants of PTC. BRAF-positive malignancies were more likely to demonstrate extrathyroidal extension ($P = .003$), lymphovascular invasion ($P = .02$), and lymph node metastasis ($P < .001$).

**Conclusions:** BRAF-positive malignant nodules most often demonstrate worrisome sonographic features and are frequently associated with positive or suspicious Bethesda cytology. In contrast, RAS-positive malignancy most often demonstrates indolent sonographic features and more commonly associates with lower risk, "indeterminate" cytology. Because BRAF and RAS mutations are the most common molecular perturbations associated with well-differentiated thyroid cancer, these findings may assist with improved preoperative risk assessment by suggesting the likely molecular profile of a thyroid cancer, even when postsurgical molecular analysis is unavailable. **(*J Clin Endocrinol Metab* 101: 4938–4944, 2016)**

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Abbreviations: FNA, fine-needle aspiration; PTC, papillary thyroid carcinoma; UG-FNA, ultrasound-guided.

Investigations have demonstrated that somatic DNA mutations and/or gene translocations of thyroid follicular cells underlie the etiology of most well-differentiated thyroid cancers (1). The most common oncogenic mutations involve the B-homologue of the rapidly accelerated fibrosarcoma (BRAF) gene, and a series of mutations involving the H, N, and K isoforms of the rat sarcoma viral oncogene homolog (RAS) gene. Together, mutations in the BRAF or RAS oncogenes can be identified in up to 75% of all well-differentiated thyroid malignancies (2). This discovery, as well as the identification of numerous other mutations/translocations responsible for the remaining cases, has led some experts to advocate for universal tissue genotyping of the newfound malignancy once a patient has been diagnosed. After identification of the responsible mutation, such knowledge may provide important prognostic information and influence treatment and/or follow-up recommendations. However, at least one published guideline does not routinely endorse this approach (3). This decision was influenced by the relative lack of available data correlating clinical, radiographic, and pathological findings with the specific mutation.

The BRAF V600E (often referred to as "BRAF-positive") mutation is the most well studied molecular mutation among well-differentiated thyroid carcinomas. Numerous investigations demonstrate that BRAF-positive papillary thyroid carcinoma (PTC) is more aggressive compared to wild-type malignancy and is also associated with higher risk PTC variants (eg, tall-cell variant) (4, 5). In univariate analysis, BRAF positivity has been independently associated with increased disease-specific mortality and recurrence (6, 7). Select other mutations involving telomerase reverse transcriptase (TERT) and tumor protein 53 (TP53) have similarly been associated with aggressive behavior, although their rare incidence has led to only limited associations. In contrast, RAS-positive mutations have primarily been associated with lower risk disease, lack of lymph node involvement, and more specifically with the follicular variant of PTC (8). Other investigations confirm that RAS mutations are also detected in a proportion of cytologically benign thyroid nodules (9, 10). Nonetheless, these and other available data provide strong evidence that specific molecular mutations associate with unique histopathological findings.

Despite this knowledge, there are few studies that provide a clear understanding of how such molecular mutations correlate with the clinical presentation of disease, or more specifically with sonographic features routinely obtained during initial evaluation. Because mutational analysis is now widely available and is utilized increasingly in clinical practice (11), such understanding may prove extremely valuable to the care of the patient. Improved risk assessment via noninvasive and minimally invasive means linking clinical and radiographic finds to the likelihood of somatic mutations would be strongly desired. Yet, to date, no such investigation has been completed.

We sought to correlate clinical, radiographic, and histopathological findings from a large group of thyroid cancer patients, with the two most common oncogenic mutations identified in well-differentiated thyroid carcinoma. Our goal was to determine whether the initial clinical evaluation and preoperative risk stratification of patients with thyroid nodular disease can ultimately be improved as clinicians are able to more accurately predict the causative oncogenic mutation.

## Subjects and Methods

As part of an ongoing prospective cohort study at the Brigham and Women's Hospital (BWH), all patients evaluated in the BWH/Dana Farber Cancer Institute (DFCI) thyroid nodule clinic undergo clinical evaluation by an endocrinologist, ultrasonographic evaluation of the thyroid by a radiologist, and fine-needle aspiration (FNA) of clinically relevant thyroid nodules as recommended by clinical guidelines current to the era of care (12, 13). Details and descriptions of this study cohort and methods of data collection have been previously published (14, 15). From this cohort, we identified patients who were referred to surgery and proved to have well-differentiated thyroid cancer. We sought permission from these patients to postoperatively perform mutational analysis of their thyroid carcinoma and identified 101 sequential patients with BRAF-positive or RAS-positive mutations. These patients are the subject of this investigation.

Molecular diagnostics were performed in the BWH Center for Advanced Molecular Diagnostics, which is a clinical laboratory certified under the Clinical Laboratory Improvement Amendment. All tumor specimens were analyzed with either the OncoMap or OncoPanel platforms, which have been previously described (16–23). These high-throughput systems use different technologies, but both are custom designed and validated to work on DNA extracted from fresh, frozen, or fixed samples. The OncoMap genotyping assay uses single nucleotide extension and chip-based MALDI mass spectrometry technology (Sequenom) to detect a total of 471 specific somatic "hotspot" mutations in 41 different genes, including all of the common mutations in the BRAF and RAS family genes (see Supplemental Data). The OncoPanel is a targeted hybridization capture-based next-generation sequencing assay that analyzes the full exonic coding sequence of 309 genes (including BRAF and the RAS family genes) plus selected intronic sequence for another 35 genes specifically interrogated for detection of structural variants, such as translocations. The OncoPanel detects any mutation in each of these genes, as well as copy number changes such as amplifications and deletions. For OncoMap, the lower limit of sensitivity was 7.5% mutant/wild type, with tumor content >30% in the specimen. For OncoPanel, the cutoff was 20% tumor content at a coverage of at least 50X. In the OncoMap system, the 471 variants are individually reported as present, absent, or no call (see Supplemental Data); OncoPanel reports only the present variants. For the purpose of this study, only RAS

and BRAF mutations that were reported as present were included.

Ultrasound examination was performed by a BWH radiologist with specific thyroid expertise. At the time of clinical assessment of the patient, nodule size (length, width, and depth), cystic component (solid, 25–75% cystic, >75% cystic), and multinodularity (two or more nodules, each >1 cm) were assessed for each patient. After surgical resection and molecular analysis, a separate radiologist blinded to all molecular analysis was provided with the size and location of the histologically proven malignant nodule. Blinded repeat interpretation of the nodule was then performed with regard to the following sonographic features: echogenicity (hypoechoic, isoechoic, hyperechoic, or mixed echogenicity), nodule margins (irregular, lobulated, or regular), calcifications (microcalcifications, rim calcifications, coarse internal calcifications, or no calcifications), radiological suspicion of extrathyroidal extension (present, absent), and the presence of abnormal lymphadenopathy in the central or lateral neck (present, absent). For several cases, complete neck imaging was performed in a separate clinic or hospital location before patients were referred for ultrasound-guided FNA (UG-FNA). For a minority of cases, these images were not available, complete, or of high enough quality for accurate interpretation. Therefore, these select cases were excluded from sonographic analysis.

Consistent with clinical recommendations contemporary to the study period, ultrasound guided fine needle aspiration (UG-FNA) was generally recommended for all noncystic nodules ≥1 cm. UG-FNA was performed with a 25-gauge needle after local anesthesia. Typically, three passes from different areas of the nodule constituted a single aspiration. Samples were processed by the ThinPrep 2000 system (Hologic Corp) and reviewed by a BWH cytopathologist. All aspirates were classified following the Bethesda System for Reporting Thyroid Cytopathology (24). Histopathology was classified using the Tumor Node Metastasis System recommended by American Joint Committee on Cancer and the World Health Organization (25, 26).

Comparisons were made using $\chi^2$ analysis or Fisher's exact test for categorical variables and with the Student's *t* test for continuous variables. Statistical analysis was performed using STATA/IC software, version 13.1 (StataCorp), and *P* values <.05 were considered significant. Approval from the BWH Office of Human Subjects Research was granted to perform this investigation.

## Results

A total of 101 sequential patients with well-differentiated thyroid carcinoma caused by mutations in the BRAF or RAS oncogenes were evaluated. Seventy-one of the 101 (72%) were BRAF-positive, whereas the remaining 30 (28%) were RAS-positive. Clinical data were available on all patients, although cytological assessment was unable to be verified in two of 101 cases. Similarly, high-quality sonographic images allowing for blinded interpretation were unavailable in 20 of 101 cases.

At presentation, RAS-positive nodules were significantly larger in size than BRAF-positive nodules (25 ± 10 vs 18 ± 13 mm, respectively; $P = .04$), although equally as likely to be a part of a multinodular gland ($P = .52$). Nearly all malignancies were solid or mostly solid. Notably, however, the sonographic characteristics of RAS-positive vs BRAF-positive nodules varied considerably at time of presentation, as shown in Table 1. Forty-four of 56 (79%) BRAF-positive nodules were hypoechoic. This was significantly different from RAS-positive nodules, in which only five of 25 nodules (20%) were hypoechoic ($P < .001$). Similarly, 26 of 56 (46%) BRAF-positive nodules had an irregular sonographic margin, compared to only four of 25 (16%) RAS-positive nodules ($P = .04$). The prevalence of microcalcifications was high in malignant nodules, although it was not significantly different between the two groups ($P = .08$). Sonographic evidence of extranodular extension and abnormal lymphadenopathy was also disproportionately noted in BRAF-positive malignancies ($P = .02$ and $P = .05$, respectively).

Details of the subject's clinical characteristics and tumor pathology are provided in Table 2. There was no statistical difference in sex distribution between RAS-positive and BRAF-positive cohorts, although RAS-positive patients were on average older at the time of presentation (56 ± 13 vs 50 ± 14 years; $P = .03$). The distribution of FNA cytology was notably different between the two cohorts. Forty-nine of 71 (69%) BRAF-positive malignancies were cytologically classified as positive for papillary carcinoma. This is in comparison to only two of 30 (7%) RAS-positive nodules ($P < .001$). In contrast, 25 of 30 (83%) RAS-positive nodules were cytologically indeterminant (atypia of undetermined significance, suspicious for a follicular or Hurthle cell neoplasm, or suspicious for papillary carcinoma). This compares to only 21 of 71 (30%) BRAF-positive nodules that were cytologically indeterminant. Furthermore, most (15 of 21, 71%) indeterminant BRAF-positive cancers were classified in the highest risk category of "suspicious for papillary carcinoma" ($P < .001$).

All BRAF-positive thyroid cancers proved to be PTCs. Although the majority (80%) of RAS-positive cancers were also PTC, four of 25 were follicular carcinomas, and one case of medullary carcinoma was identified. Notably, the distribution of PTC variants differed markedly between groups. Twenty-four of 25 (96%) RAS-positive PTCs were follicular variants of PTC. In contrast, only 12 of 71 (17%) BRAF-positive malignancies were follicular variants of PTC ($P < .001$). Instead, most BRAF-positive malignancies were classical variants of PTC (50 of 71, 70%), with a separate minority classified as tall cell variants (8 of 71, 12%)

Histological features of aggressive malignant behavior were more commonly visualized in BRAF-positive malig-

**Table 1.** Comparison of Sonographic Features of Malignant Nodules by Mutation Type

| | RAS-Positive Malignancy | BRAF-Positive Malignancy | *P* Value |
|---|---|---|---|
| n | 25 | 56 | |
| Largest single dimension (mean ± SD | 25 mm ± 10 mm | 18 mm ± 13 mm | **.04** |
| No. of nodules | | | .52 |
| Solitary nodule | 15 (60) | 29 (52) | |
| Part of multinodular goiter | 10 (40) | 27 (48) | |
| Cystic content of nodule | | | .65 |
| <25% cystic | 23 (96) | 53 (95) | |
| 25–75% cystic | 2 (4) | 3 (5) | |
| >75% cystic | 0 | 0 | |
| Parenchyma | | | **<.001** |
| Hypoechoic | 5 (20) | 44 (79) | |
| Isoechoic | 11 (44) | 10 (18) | |
| Hyperechoic | 1 (4) | 1 (1.5) | |
| Mixed | 8 (32) | 1 (1.5) | |
| Margins | | | **.04** |
| Irregular | 4 (16) | 26 (46) | |
| Lobulated | 5 (20) | 7 (13) | |
| Regular | 16 (64) | 23 (41) | |
| Calcifications[a] | | | |
| Microcalcification | 21 (84) | 35 (63) | .08 |
| Rim calcification | 1 (4) | 6 (11) | .43 |
| Coarse calcification | 6 (24) | 19 (34) | .44 |
| More tall than wide | 0 (0) | 6 (11) | .17 |
| Radiographic findings of extranodular extension | 1 (4) | 16 (29) | **.02** |
| Abnormal lymph nodes present | 0 (0) | 9 (17) | .05 |

Data are expressed as number (percentage), unless designated otherwise.

[a] Each calcification finding is not mutually exclusive because both microcalcification and/or rim or coarse calcification can occur together. Thus, each variable is reported separately.

nancies. Twenty-one of 71 (30%) BRAF-positive malignancies demonstrated extrathyroidal extension, compared to only one of 30 (3%) RAS-positive malignancies ($P = .003$). Similar disparities were noted with regard to lymphovascular invasion ($P = .02$) and the presence of local lymph node metastasis ($P < .001$), although not for the presence of distant metastatic disease ($P = .99$).

## Discussion

Although the identification of the molecular underpinnings of malignant thyroid disease is now possible, its translation, meaning, and clinical impact remain less certain. Our data reveal new associations between the identified oncogenic mutation and a nodule's preoperative sonographic assessment and FNA cytology. RAS-positive malignancies are most often cytologically indeterminate and frequently lack sonographic features concerning for malignancy. In contrast, BRAF-positive malignancies are most often cytologically positive for PTC and commonly demonstrate sonographic features of hypoechogenicity, microcalcifications, and irregular margins. Together, these data demonstrate that thyroid malignancy is a diverse entity, while also demonstrating that different oncogenic pathways modulate the clinical, radiological, and histological presentation of disease.

Mutations in the BRAF oncogene strongly associate with PTC and are often correlated with more aggressive and metastatic disease (4–7). But the phenotype of RAS-positive thyroid cancer has been less well defined. Some have reported high rates of RAS mutations in advanced metastatic thyroid carcinoma (27), whereas others increasingly associate RAS-positive malignancy with more indolent disease (8). Still others have also identified RAS mutations in benign follicular adenomas, without evidence of malignant transformation over time (9, 10). Our data support the latter. Nearly 50% of BRAF-positive malignancies in our series demonstrated local metastatic disease, with 46 and 30%, respectively, exhibiting lymphovascular invasion and extrathyroidal extension. This was notably different from RAS-positive thyroid cancer, in which no lymph node disease (0%) was identified and only one case exhibited extrathyroidal extension. It is also notable that RAS-positive thyroid cancer was generally larger upon presentation in comparison to BRAF-positive thyroid cancer, while also detected in older patients. This suggests that RAS mutations may stimulate clonal growth of follicular thyroid cells, but without other necessary

**Table 2.** Comparison of Patient Characteristics, FNA Cytology, and Histopathology by Mutation Type

| | RAS-Positive Malignancy | BRAF-Positive Malignancy | *P* Value |
|---|---|---|---|
| No. of subjects | 30 | 71 | |
| Females | 21 (70) | 58 (82) | .19 |
| Age at nodule presentation, y | | | |
| Mean ± SD | 56 ± 13 | 50 ± 14 | **.03** |
| Range | 29–76 | 19–71 | |
| FNA cytology | | | |
| Benign | 1[a] (3) | 0 | **<.001** |
| AUS/FLUS | 8 (28) | 5 (7) | |
| FN/SFN | 9 (31) | 1 (1) | |
| Suspicious for PTC | 8 (28) | 15 (22) | |
| PTC | 2 (7) | 49 (70) | |
| Medullary thyroid carcinoma | 1 (3) | 0 | |
| Not available[b] | 1 | 1 | |
| Histology | | | **.002** |
| PTC | 25 (83) | 71 (100) | |
| Follicular thyroid carcinoma | 4 (14) | 0 | |
| Medullary thyroid carcinoma | 1 (3) | 0 | |
| PTC histological variant | | | **<.001** |
| Classical | 1 (4) | 50 (70) | |
| Follicular | 24 (96) | 12 (17) | |
| Tall cell | 0 | 8 (12) | |
| Hobnail | 0 | 1 (1) | |
| Histological findings | | | |
| Extrathyroidal extension | 1 of 30 (3) | 21 of 71 (30) | **.003** |
| Lymphovascular invasion | 6 of 30 (20) | 31 of 71 (46) | **.02** |
| Lymph node metastasis | 0 of 30 (0) | 35 of 71 (49) | **<.001** |
| Distant metastasis | 0 of 30 (0) | 2 of 71 (3) | .99 |

Abbreviations: AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; FN, follicular neoplasm, SFN, suspicious for follicular neoplasm. Data are expressed as number (percentage), unless stated otherwise.

[a] Malignancy was a 1.4-cm follicular variant PTC arising in a 4.5-cm follicular adenoma. Benign tissue was felt to represent the majority of tissue volume.

[b] Samples not included in percentage calculations.

changes that stimulate malignant behavior. Hypothetically, a separate or secondary molecular "hit" may be necessary for RAS-positive nodules to transform into more malignant disease.

Ultrasound evaluation of the thyroid is the standard recommended approach for thyroid nodule evaluation (3). This is largely because ultrasound is superior to palpation in its ability to accurately detect the extent of disease while also improving the accuracy of FNA. More recently, however, further support for sonographic analysis of nodules is supported by a wealth of investigations confirming the ability of sonographic assessment to meaningfully predict cancer risk (28). In doing so, the clinician can then preferentially aspirate nodules with high-risk features such as hypoechoic parenchyma, microcalcifications, and/or irregular borders. Our data support these findings for BRAF-positive malignancies, but also raise newfound concerns surrounding RAS-positive disease. Only 20% of RAS-positive thyroid cancers were found to have hypoechoic parenchyma, and only 16% demonstrated irregular sonographic margins. This is in stark contrast to nearly 80 and 46% of BRAF-positive malignancies that demonstrated hypoechoic parenchyma or irregular margins, respectively. A notable finding was that microcalcifications were commonly seen in both RAS-positive and BRAF-positive thyroid cancers, by far depicting the most common sonographic abnormality identified. These findings suggest that ultrasound may prove less sensitive in detecting all RAS-positive thyroid cancer, although the downstream impact of this is uncertain. If RAS-positive cancer has lower risk and is at times identified in benign disease, delaying FNA or diagnosis may not prove harmful. In our series, the findings of solid (noncystic) parenchyma with microcalcifications proved most specific for well-differentiated thyroid cancer of either type.

We acknowledge limitations to our investigation. Mutational analysis was performed only on malignant nodules. Thus, our understanding of the molecular profile for benign or borderline (eg, noninvasive, follicular thyroid neoplasm with papillary-like nuclear features nodules) remains incomplete. Because of this, our findings do not provide complete guidance for any sonographic-molecular correlation in a broad population of patients presenting with nodular disease. Such a study, however, would be

very large and is likely to be prohibitively costly to perform. We also note that preoperative FNA cytology can influence the extent of initial recommended thyroid surgery. This, in turn, can then impact the proportion of local lymph node metastatic disease identified. However, because preoperative ultrasound was performed on the entire cohort, it is unlikely that clinically relevant lymph node disease would go undetected.

Together, these data provide guidance for practicing clinicians as they evaluate patients with thyroid nodular disease because BRAF and RAS mutations are the two most common molecular perturbations causing well-differentiated thyroid cancer. Nodules with worrisome sonographic features and either malignant or suspicious Bethesda cytology are more likely to prove BRAF-positive than RAS-positive if malignant. In contrast, nodules with indolent sonographic findings and a preceding indeterminate cytology are much more likely to be RAS-positive than BRAF-positive if malignant. Even if postsurgical molecular analysis is not performed, the above associations allow better understanding of overall risk and the likely molecular profile. From this, improved prognostic and follow-up strategies can be created. In an era where individualized care is paramount to medical care, such associations allow even further personalization. Such knowledge, in combination with other clinical factors, may notably influence the decision to perform a hemithyroidectomy vs near-total resection. Importantly, however, the field has not yet elucidated the full spectrum of molecular profiles in benign thyroid nodules. Therefore, it is impossible to accurately determine the predictive value of any sonographic features with regard to molecular mutations.

In summary, whereas RAS and BRAF mutations appear to be the critical molecular processes underpinning most well-differentiated thyroid cancer, their clinical-sonographic-pathological profiles differ dramatically. Our data depict these important associations and provide helpful data because practicing clinicians are increasingly called upon to integrate molecular findings into clinical practice and deliver personalized care to their patients.

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