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NRAS Q61R Immunohistochemical Staining in Thyroid Pathology: Sensitivity, Specificity and Utility

Maelle Saliba, **Nora Katabi**, **Snjezana Dogan**, **Bin Xu***, **Ronald A. Ghossein***

Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Background: The diagnosis of thyroid neoplasms relies on the demonstration of histologic parameters that can be focal and prone to subjective interpretation. We evaluated the utility of NRAS Q61R immunohistochemistry (IHC) in the diagnosis of thyroid lesions after determining its specificity and sensitivity as a surrogate marker for *RAS* Q61R mutation.

Design: NRAS Q61R IHC was performed on 282 primary or metastatic thyroid lesions from 256 patients. *RAS* mutation status was collected from patients' chart. Sensitivity and specificity of NRAS Q61R IHC for detecting a *RAS* Q61R mutation was calculated. IHC-positive cases were reviewed to determine the diagnostic utility of NRAS Q61R IHC.

Results: NRAS Q61R immunopositivity was seen in non-neoplastic, benign, and malignant thyroid lesions. NRAS Q61R antibody cross-reactivity led to the detection of NRAS Q61R, KRAS Q61R and HRAS Q61R proteins. Amongst primary thyroid carcinomas, immunopositivity was most frequent in papillary thyroid carcinomas, follicular variant (48.0%). The sensitivity and specificity of NRAS Q61R IHC in detecting *RAS* Q61R mutation was 90.6% and 92.3%, respectively. When positive, the NRAS Q61R stain was determined to be helpful in demonstrating infiltration, tumor size, capsular and/or vascular invasion and multifocality.

Conclusion: NRAS Q61R IHC is highly sensitive and specific for the detection of *RAS* Q61R mutations in thyroid pathology and is particularly relevant in follicular-patterned neoplasms. When evaluated alongside histologic features, NRAS Q61R immunoreactivity can be instrumental in the diagnosis and classification of thyroid nodules.

Graphical Abstract

* Co-senior corresponding authors: Ronald Ghossein, MD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York, 10065, ghosseir@mskcc.org; Bin Xu MD, PhD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York, 10065, Xub@mskcc.org.

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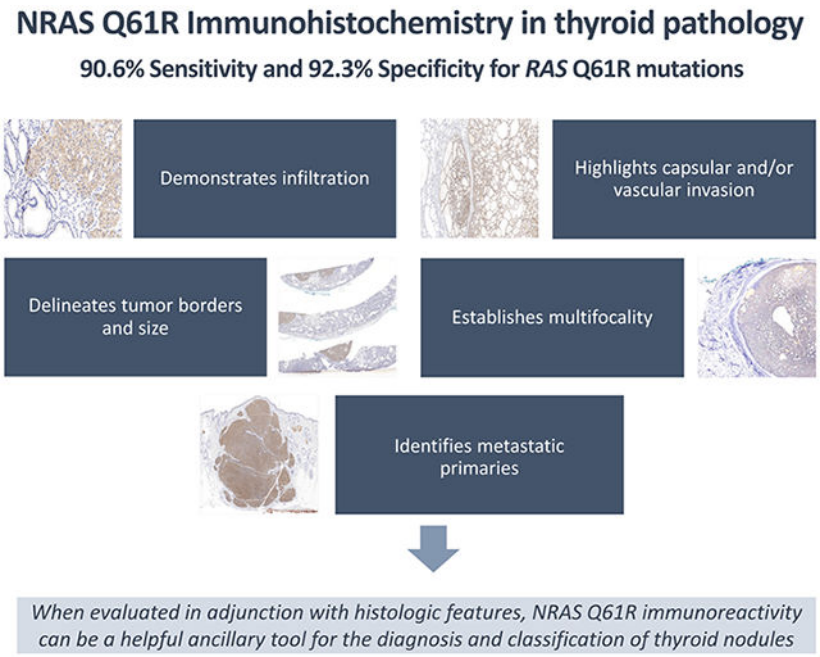

Keywords

NRAS Q61R; immunohistochemistry; thyroid neoplasms; papillary thyroid carcinoma

Introduction

Thyroid pathology has witnessed multiple paradigm shifts in the last few decades. More than ever, the diagnosis and subtyping of various thyroid lesions relies on the demonstration of histologic features that can be focal and prone to subjective interpretation. To cite a few examples, the diagnosis of noninvasive follicular thyroid tumor with papillary-like nuclear features (NIFTP) requires the demonstration of well-demarcated lesional borders with no surrounding parenchymal infiltration.[1] The diagnosis of follicular thyroid carcinoma (FTC) is based on the identification of capsular and/or vascular invasion, sometimes amidst a background of follicular-patterned thyroid pathology such as nodular hyperplasia.[2] Lastly, a satellite tumor nodule with a histologic appearance that differs from that of the main lesion may present a diagnostic dilemma in the sense that it may represent either capsular invasion from the main nodule or tumor multicentricity. The unequivocal demonstration of these histopathologic features can sometimes be problematic, and ancillary testing is often needed as a valuable complement to hematoxylin and eosin (H&E). In that regard, mutation-specific immunohistochemistry (IHC) can be instrumental in evaluating genetic mutations at the protein level in neoplasia.[3]

The oncogenic *NRAS* c.182A>G mutation, an activating mutation of *RAS* oncogene, is a major driver mutation in follicular-patterned thyroid neoplasms and results in the NRAS Q61R protein.[4, 5] The NRAS Q61R IHC has recently been developed and is now commercially available, mainly for its use in malignant melanomas, but also in colorectal

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carcinomas and breast adenomyoepitheliomas.[6-9] Though its sensitivity and specificity have been addressed in a few publications, its exact diagnostic and clinical utility in thyroid pathology has yet to be fully established.[5, 10]

The first goal of this project was to assess the reliability, sensitivity and specificity of IHC in identifying *RAS* Q61R mutations in thyroid lesions. The second was to explore the diagnostic utility of *NRAS* Q61R mutation-specific antibody as an adjunct to histologic interpretation.

Methods

Study cohort and histopathology

The study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY). A total of 282 primary or metastatic thyroid lesions from 256 patients diagnosed from 1994 to 2020 were included. The study cohort consisted of 11 non-neoplastic lesions (10 hyperplastic nodules (HNs) and 1 thyroid remnant), 9 benign neoplasms (5 follicular thyroid adenomas (FTAs), 3 Hurthle cell adenomas (HCAs) and 1 hyalinizing trabecular tumor), 67 NIFTPs, 183 primary carcinomas (148 papillary thyroid carcinomas (PTC), 16 poorly differentiated thyroid carcinomas (PDTCs), 7 FTCs, 6 anaplastic thyroid carcinomas and 6 Hurthle cell carcinomas) and 12 metastases from thyroid carcinomas. The thyroid carcinomas were classified according to the last World Health Organization classification of endocrine tumors, except for PDTCs, for which MSKCC criteria were used.[11] As per the latter, the tumor was diagnosed as PDTC if it displayed high mitotic activity (≥5 mitosis/10 high-power fields, ×400 corresponding to 2.4 mm2) and/or tumor necrosis and showed follicular cell differentiation at the morphologic or IHC level.[12] Follicular variant PTCs (FVPTC) were classified according to the tumor's two major subtypes: infiltrative FVPTC (displaying obvious and significant infiltration of the normal thyroid with accompanying fibrotic reaction) and encapsulated/well demarcated FVPTC showing histologic evidence of invasion. The latter subtype was further subdivided into encapsulated FVPTC showing capsular and/or vascular invasion (figure 1) and well-demarcated (unencapsulated) FVPTC with minimal infiltration (percolation of a few (<5) neoplastic follicles in between non-neoplastic ones with no fibrotic reaction) (figure 2).

BRAFV600E and NRAS Q61R immunohistochemistry

All cases included in this study underwent NRAS Q61R IHC. A limited number of lesions were also immunostained for BRAF V600E, at the discretion of the pathologist at the time of diagnosis. IHC staining for BRAF V600E and NRAS Q61R was performed with an anti-BRAFV600E monoclonal antibody (clone: VE1, dilution: 1:400; Abcam, Cambridge, MA, USA), and an anti-NRAS Q61R monoclonal antibody (clone: SP174, dilution: 1:25; Abcam, Cambridge, MA, USA) using the Leica Bond III system (Leica Biosystems, Inc., Buffalo Grove, IL, USA) according to the manufacturer's recommendations.

IHC interpretation

A panel of three endocrine pathologists (R.G., B.X. and M.S.) blinded to the genotyping results scored the NRAS Q61R stains. Staining intensity was scored as 0 (no staining), 1+

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(weak), 2+ (moderate), and 3+ (strong). The NRAS Q61R IHC was interpreted as positive if it exhibited cytoplasmic and/or membranous immunoreactivity with a staining intensity of 2+ or 3+ irrespective of the number of tumor cells stained (figure 3). Homogeneous expression was defined as analogous labeling intensity of 2-3+ in ≥80% of the tumor cells. BRAF V600E IHC was interpreted as positive if it displayed a staining intensity of 2+ or 3+ irrespective of the number of tumor cells stained. The H&E and IHC slides were reviewed by the panel of pathologists to assess the utility of NRAS Q61R IHC (expressed as either helpful or uninformative in providing the following information: highlighting tumor borders, confirming invasion, determining multifocality, demarcating the lesion and confirming thyroid origin of a metastasis).

Molecular analysis and diagnostic test characteristics calculation

RAS mutation status was collected from patients' charts when available. The sensitivity, specificity, positive and negative predictive values of NRAS Q61R IHC for detecting a *RAS* Q61R mutation were calculated.

Results

NRAS Q61R IHC expression

Table 1 summarizes the NRAS Q61R staining frequency in each of the evaluated diagnostic categories. Overall, the positive expression of NRAS Q61R was observed in 29.1% of cases. All the positive cases exhibited homogeneous moderate-to-strong granular cytoplasmic and/or membranous staining (2 to 3+/3) in at least 90% of the lesional cells. In some cases, the background follicular cells focally displayed weak inhomogeneous staining, easily differentiated from the stronger homogeneous staining seen in the lesional cells.

RAS mutational status

Data on RAS mutation status was available on 84 cases. The testing platforms consisted of ThyroSeq® Genomic Classifier (University of Pittsburgh Medical Center, Pittsburgh, PA, USA) in 44 cases, MSK-IMPACT™ (MSKCC, New York, NY, USA) in 22 cases, Sequenom-based genotyping assay (Sequenom mass array; Sequenom, San Diego, California, USA) in 9 cases, ThyGeNEXT® + ThyraMIR® Thyroid Oncogene Panel (InterpaceDx lab, Pittsburgh, PA, USA) in 7 cases, Afirma Xpression Atlas (Veracyte, South San Francisco, California, USA) in 1 case, and Asuragen's miRInform Thyroid test (Asuragen Inc, Austin, TX, USA) in 1 case. Table 1 summarizes *RAS* Q61R and *NRAS* Q61R mutation frequency in each of the evaluated diagnostic categories.

Correlation of NRAS Q61R IHC expression with *RAS/HRAS/KRAS* mutation status

The relationship between NRAS Q61R immunoexpression and the molecular genotypes of these cases is summarized in Table 2. The sensitivity and specificity of NRAS Q61R IHC staining for the *RAS* Q61R mutation in all assessed cases were 90.6% and 92.3%, respectively. The positive and negative predictive values were 87.9% and 94.1%, respectively. The sensitivity and specificity of NRAS Q61R IHC staining for the *NRAS* Q61R mutation in all assessed cases were 90.0% and 76.6%, respectively. Eighteen of 33 lesions positive for NRAS Q61R IHC exhibited the *NRAS* Q61R mutation. The remaining

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15 lesions showed either a *HRAS* Q61R mutation (7 cases), a *KRAS* Q61R mutation (4 cases) or did not harbor a *RAS* mutation (4 cases). The intensity of NRAS Q61R immunoreactivity did not change according to which *RAS* oncogene was mutated. No IHC labeling was observed in the 11 cases with mutations at codon 61 of *KRAS* or *NRAS* that showed amino acid alterations other than Q61R. The four false positive cases consisted of two NIFTPs, one PDTC and one HCA. Three false negative cases consisted of one *NRAS* Q61R-mutated encapsulated FVPTC, one *HRAS* Q61R-mutated encapsulated FVPTC and one *NRAS* Q61R-mutated PDTC.

Clinical utility

When positive, the NRAS Q61R stain was determined to be helpful in the pathologic diagnosis of thyroid lesions in 86.4% of cases, alternatively aiding in highlighting infiltration (n=11) or well-circumscribed (n=31) tumor borders, identifying satellite nodules as capsular invasion (n=21), confirming vascular invasion (n=1), determining multifocality (n=3), demarcating the entirety of the lesion for accurate size assessment (n=2) and confirming the thyroid origin of a metastasis (n=1).

Discussion

Antibodies specific for mutated amino acid sequences are the subject of increased interest in diagnostic pathology practice.[13] Unlike molecular testing, they provide rapid, accessible, cost-efficient, and reliable means of detecting molecular aberrations.[3, 8] They require less preparation and can be interpreted by general pathology practitioners.[3] BRAF V600E IHC has already set a precedent for the use of mutation-specific antibodies as an adjunct to H&E interpretation across multiple tumor types, including thyroid lesions.[14-20] SP174, the antibody directed against RAS Q61R, is a recently developed marker made commercially available mainly for its use in malignant melanoma, in which the antibody is reported to have an excellent specificity and sensitivity.[6, 21, 22] A limited number of studies have already addressed the reliability of NRAS Q61R IHC in thyroid pathology, however the marker has yet to be integrated in routine practice.[5, 10, 23, 24] Our findings demonstrate that SP174 labels not only *NRAS* Q61R-mutated tumors, but also cross-reacts with mutant KRAS Q61R and HRAS Q61R proteins. This cross-reaction is established in the literature and has proven to be useful in routine practice.[8, 24, 25]

Among *RAS* mutations, the substitution at codon 61 of *NRAS*, which leads to NRAS Q61R, is the most common type.[5] The same substitution can also involve the *HRAS* and *KRAS* genes.[8] Activating point mutations of the *RAS* gene are not specific to a particular thyroid tumor histotype, but are rather associated with follicular-patterned lesions, and are thus found in FTAs (20-48%),FTCs (49%), HCCs (9-15%), encapsulated FVPTCs and NIFTPs (30-52%), PDTCs (45%) and ATCs (23%).[4, 26-28] RAS Q61R IHC is therefore particularly pertinent in FVPTC, which harbors the highest prevalence of *RAS* mutations amongst papillary thyroid carcinomas.[2] A significant percentage of the FVPTC in our study showed positive labeling with SP174 (48.0%) and *RAS* Q61R gene mutations (54.0%). This is consistent with the literature, which associates FVPTC with the RAS-like molecular group of MAPK pathway activated thyroid neoplasia.[29] As predicted, we found encapsulated

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FVPTCs to be enriched in *RAS* mutations (NRAS Q61R immunoreactivity in 47.5% and *RAS* Q61R mutations in 53.8%).[30] Well-demarcated FVPTCs with minimal parenchymal infiltration also displayed frequent positive NRAS Q61R IHC labeling (58.3%) (figure 2) indicating a similar molecular profile to that of encapsulated FVPTC with capsular/vascular invasion. Well-demarcated FVPTC with minimal infiltration thus seems to differ from obviously infiltrative FVPTC, which harbors a much lower rate of RAS mutations. [26, 30] In other terms, it seems that while obvious infiltration is associated with *BRAF* V600E mutations, limited infiltration in FVPTC is associated with *RAS* mutations.[30]

The current study agrees with previous reports in that the SP174 antibody is a highly sensitive and specific surrogate marker for *RAS* Q61R mutations in thyroid pathology, with a sensitivity and specificity reaching 90%. SP174 is therefore a reasonable tool for the detection of these genetic alterations in thyroid lesions, especially in instances where genotyping is not available or when time is of the essence. Of note, both Oishi et al. and Crescenzi et al. had previously reported a sensitivity of 100%.[5, 10] Our lower sensitivity figure could be due to our higher threshold in regarding a certain staining intensity as positive. As for our apparent false positive cases, one explanation could be the imperfect sensitivity of the platforms we relied on to detect *RAS* Q61R mutations. Two of the false positive cases were genotyped using the Sequenom-based genotyping assay, which is known to have lower sensitivity than other methods.[31]

Based on the fact that *RET* and *HRAS* Q61R mutations are mutually exclusive, SP174 antibody may have a role in triaging which medullary thyroid carcinoma patients receive germline testing for *RET* protooncogene.[32, 33] Aside for this possible role in circumventing costly molecular testing, the clinical utility of RAS Q61R IHC in thyroid pathology was not thoroughly discussed in the literature. [5, 10]

As shown in our cohort and in the literature, both non-neoplastic (i.e. HN) and benign neoplasms (e.g. FTAs) can label positively for SP174 and harbor *RAS* Q61R gene mutations.[34] The traditional thinking adopted by some authors is that HNs are non-neoplastic, polyclonal lesions, while FTAs represent clonal benign neoplasms.[35] This opinion, however, is not shared by all, and many authors believe that lesions histologically diagnosed as HNs could be clonal despite being non-neoplastic.[36] As in Oishi et al.'s study, 20% of HNs in our cohort homogeneously expressed the mutant RAS Q61R protein, proving that the term HN currently serves as morphologic umbrella for lesions that could very well be either mono or polyclonal.[5] Overall, the preoperative finding of a *RAS* mutation portends a 40 to 80% risk of malignancy if NIFTP is considered malignant, and a malignancy risk of 25% excluding NIFTP.[34, 37] Based on the above, RAS Q61R IHC cannot be used to diagnose malignancy that is not demonstrated histologically. Rather, its diagnostic utility can be used in several circumstances where the histologic features of a *RAS* Q61R-mutated lesion are not conclusive or could benefit from further affirmation.

Highlighting infiltration

In the first of these instances, positive labeling for NRAS Q61R IHC helped demonstrate subtle infiltration into non-neoplastic thyroid follicles (figure 2). The stain highlighted the presence of NRAS Q61R immunoreactive neoplastic follicles permeating in between non-

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neoplastic NRAS negative background thyroid follicles. As such, the antibody facilitated the diagnosis of well-demarcated FVPTC with minimal infiltration. Alternatively, NRAS Q61R helped confirm the absence of any infiltration or invasion in cases with unencapsulated tumors with a seemingly well-circumscribed border. These cases were labeled as either NIFTP or noninvasive well-circumscribed/encapsulated PTC when the lack of infiltration was confirmed but NIFTP criteria were not met.[1, 38] In that regard, the stain helped differentiate NIFTP from invasive PTC in follicular patterned tumors.

Highlighting capsular invasion

As in Oishi et al.'s study, several of our cases exemplified how NRAS Q61R IHC can help identify capsular invasion by highlighting both the *RAS* Q61R-mutated main nodule and the suspicious focus.[5] The stain thus facilitates the visualization of a permeating lesional bud. It also provides valuable information in cases with satellite nodules situated further away from the outer aspect of the capsule and lacking the classic mushroom-shaped bud (figure 1).

Demonstrating multifocality

Conversely, NRAS Q61R can be useful in demonstrating multifocality when faced with multiple adjacent foci of tumor. Similar NRAS Q16R immunopositivity in two adjacent tumor foci is a strong argument in favor of the satellite nodules being part of the main nodule. In contrast, divergent immunohistochemical staining profiles support the presence of separate neoplasms with different driver mutations (figure 4).

Accentuating vascular invasion

NRAS Q61R IHC can also be combined with vascular markers such as CD31 to accentuate the presence of vascular invasion by highlighting the presence of intravascular NRAS Q61R positive lesional cells, as noted by Oishi et al.[5]

Delineating the tumor

The immunohistochemical detection of gene mutations using mutation-specific antibodies provides the ability to delineate the surface area of a nodule by allowing the direct visualization of the mutant protein's expression in situ.[39] This offers valuable insight into the exact measurement of the tumor, thus preventing the under or overestimation of its greatest dimension and is especially important when the tumor size hovers around clinically significant cutoffs. A particular vexing situation occurs when the neoplastic process arises amidst a larger lesion, the best example being a FVPTC arising within a HN. Visualizing the clonal *NRAS* mutant process and separating it from the non-neoplastic follicles prevents interpreting the whole nodule as a higher stage tumor (figure 5). The opposite also applies, as NRAS Q61R IHC can help prevent underestimating the total size of a tumor. Because thyroid carcinoma can show an intratumoral spectrum of architectural and nuclear features, visualizing the shared mutant protein expression can help bridge the gap created by phenotypic heterogeneity.

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Matching a metastasis with a thyroid primary

Lastly, SP174 can be instrumental in matching a distant metastasis with a *RAS* Q61R-mutated thyroid primary. Considering that oncogenic *RAS* Q61R mutations are found in a great variety of human cancers, transcriptional evidence of the mutation is not, in itself, specific to thyroid carcinomas.[40] However, in the appropriate clinical context, and when interpreted alongside a panel of relevant IHC markers such as PAX8, TTF-1 and thyroglobulin, NRAS Q61R positivity in both the primary and the metastasis can serve as an additional argument in favor of a thyroid origin (figure 6). This is most helpful in metastatic anaplastic carcinomas that show loss of TTF1 and PAX8 expression.[41] In instances with multifocal thyroid carcinomas and an NRAS Q61R immunoreactive distant metastasis, using NRAS Q61R immunohistochemistry may help identify the metastatic primary for the purpose of genotyping the metastasizing tumoral process (figure 7).

Conclusion

NRAS Q61R is a highly sensitive and specific antibody for the detection of the *RAS* Q61R mutations in thyroid pathology. When evaluated alongside histologic features , it can be helpful in multiple instances, whether it is by aiding in highlighting infiltrative or well-circumscribed tumor borders, by confirming capsular or vascular invasion, by determining multifocality, by demarcating the entirety of the lesion for accurate size assessment or by confirming the thyroid origin of a metastasis. Considering that *RAS* Q61R mutations are mutually exclusive with other mutations in the MAPK pathway, SP174 antibody may one day have a role in triaging which patients with aggressive follicular cell derived thyroid carcinomas need additional molecular testing or may benefit from targeted therapy, as is currently done based on BRAFV600E and ALK immunohistochemical stains.[42, 43]

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References

1. Xu B, Ghossein RA. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (niftp): An update. Head Neck Pathol2020;14;303–310. [PubMed: 32124418]
2. Ghossein REncapsulated malignant follicular cell-derived thyroid tumors. Endocr Pathol2010;21;212–218. [PubMed: 21088998]
3. Sukswai N, Khoury JD. Immunohistochemistry innovations for diagnosis and tissue-based biomarker detection. Curr Hematol Malig Rep2019;14;368–375. [PubMed: 31338668]
4. Nikiforov YE. Molecular analysis of thyroid tumors. Mod Pathol2011;24Suppl 2;S34–43. [PubMed: 21455199]
5. Oishi N, Kondo T, Vuong HGet al.Immunohistochemical detection of nras(q61r) protein in follicular-patterned thyroid tumors. Hum Pathol2016;53;51–57. [PubMed: 26980032]
6. Massi D, Simi L, Sensi Eet al.Immunohistochemistry is highly sensitive and specific for the detection of nrasq61r mutation in melanoma. Mod Pathol2015;28;487–497. [PubMed: 25341653]

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7. Turchini J, Andrici J, Sioson Let al.Nrasq61r mutation-specific immunohistochemistry is highly specific for either nrasq61r or krasq61r mutation in colorectal carcinoma. Appl Immunohistochem Mol Morphol2017;25;475–480. [PubMed: 26862952]
8. Jhuang JY, Yuan CT, Lin YL, Cheng ML, Liau JY, Tsai JH. Nrasq61r immunohistochemistry detects both nrasq61r and krasq61r mutations in colorectal cancer. Pathology2017;49;387–390. [PubMed: 28433252]
9. Pareja F, Toss MS, Geyer FCet al.Immunohistochemical assessment of hras q61r mutations in breast adenomyoepitheliomas. Histopathology2020;76;865–874. [PubMed: 31887226]
10. Crescenzi A, Fulciniti F, Bongiovanni M, Giovanella L, Trimboli P. Detecting n-ras q61r mutated thyroid neoplasias by immunohistochemistry. Endocr Pathol2017;28;71–74. [PubMed: 28064410]
11. Lloyd RV, Osamura RY, Kloppel G, Rosai J. Who classification of tumours of endocrine organs. Fourth edition ed, 2017.
12. Hiltzik D, Carlson DL, Tuttle RMet al.Poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis: A clinicopathologic study of 58 patients. Cancer2006;106;1286–1295. [PubMed: 16470605]
13. Uguen A, Talagas M, Costa Set al.Nras(q61r), braf(v600e) immunohistochemistry: A concomitant tool for mutation screening in melanomas. Diagn. Pathol2015;10;10. [PubMed: 25889528]
14. Loo E, Khalili P, Beuhler K, Siddiqi I, Vasef MA. Braf v600e mutation across multiple tumor types: Correlation between DNA-based sequencing and mutation-specific immunohistochemistry. Appl Immunohistochem Mol Morphol2018;26;709–713. [PubMed: 29271794]
15. Capper D, Preusser M, Habel Aet al.Assessment of braf v600e mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. Acta Neuropathol2011;122;11–19. [PubMed: 21638088]
16. Long GV, Wilmott JS, Capper Det al.Immunohistochemistry is highly sensitive and specific for the detection of v600e braf mutation in melanoma. Am J Surg Pathol2013;37;61–65. [PubMed: 23026937]
17. Capper D, Berghoff AS, Magerle Met al.Immunohistochemical testing of braf v600e status in 1,120 tumor tissue samples of patients with brain metastases. Acta Neuropathol2012;123;223–233. [PubMed: 22012135]
18. Ilie MI, Lassalle S, Long-Mira Eet al.Diagnostic value of immunohistochemistry for the detection of the braf(v600e) mutation in papillary thyroid carcinoma: Comparative analysis with three DNA-based assays. Thyroid2014;24;858–866. [PubMed: 24417277]
19. Zagzag J, Pollack A, Dultz Let al.Clinical utility of immunohistochemistry for the detection of the braf v600e mutation in papillary thyroid carcinoma. Surgery2013;154;1199–1204; discussion 1204–1195. [PubMed: 23931769]
20. Bullock M, O'Neill C, Chou Aet al.Utilization of a mab for braf(v600e) detection in papillary thyroid carcinoma. Endocr Relat Cancer2012;19;779–784. [PubMed: 22997209]
21. Ilie M, Long-Mira E, Funck-Brentano Eet al.Immunohistochemistry as a potential tool for routine detection of the nras q61r mutation in patients with metastatic melanoma. J. Am. Acad. Dermatol2015;72;786–793. [PubMed: 25659223]
22. Uguen A, Talagas M, Costa Set al.Nras (q61r), braf (v600e) immunohistochemistry: A concomitant tool for mutation screening in melanomas. Diagn Pathol2015;10;121. [PubMed: 26204954]
23. Dias-Santagata D, Su Y, Hoang MP. Immunohistochemical detection of nrasq61r mutation in diverse tumor types. Am J Clin Pathol2016;145;29–34. [PubMed: 26712868]
24. Xu B, Serrette R, Tuttle RMet al.How many papillae in conventional papillary carcinoma? A clinical evidence-based pathology study of 235 unifocal encapsulated papillary thyroid carcinomas, with emphasis on the diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features. Thyroid2019;29;1792–1803. [PubMed: 31452453]
25. Felisiak-Gołąbek A, Inaguma S, Kowalik Aet al.Sp174 antibody lacks specificity for nras q61r and cross-reacts with hras and kras q61r mutant proteins in malignant melanoma. Appl Immunohistochem Mol Morphol2018;26;40–45. [PubMed: 29206715]
26. Haroon Al Rasheed MR, Xu B. Molecular alterations in thyroid carcinoma. Surg Pathol Clin2019;12;921–930. [PubMed: 31672298]

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27. Xing MClinical utility of ras mutations in thyroid cancer: A blurred picture now emerging clearer. BMC Med2016;14;12. [PubMed: 26817707]
28. Howell GM, Hodak SP, Yip L. Ras mutations in thyroid cancer. Oncologist2013;18;926–932. [PubMed: 23873720]
29. Nikiforov YE. Thyroid carcinoma: Molecular pathways and therapeutic targets. Mod Pathol2008;21Suppl 2;S37–43. [PubMed: 18437172]
30. Rivera M, Ricarte-Filho J, Knauf Jet al.Molecular genotyping of papillary thyroid carcinoma follicular variant according to its histological subtypes (encapsulated vs infiltrative) reveals distinct braf and ras mutation patterns. Mod Pathol2010;23;1191–1200. [PubMed: 20526288]
31. Arcila M, Lau C, Nafa K, Ladanyi M. Detection of kras and braf mutations in colorectal carcinoma roles for high-sensitivity locked nucleic acid-pcr sequencing and broad-spectrum mass spectrometry genotyping. J Mol Diagn2011;13;64–73. [PubMed: 21227396]
32. Agrawal N, Jiao Y, Sausen Met al.Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in ret and ras. J Clin Endocrinol Metab2013;98;E364–369. [PubMed: 23264394]
33. Reagh J, Bullock M, Andrici Jet al.Nras(q61r) mutation-specific immunohistochemistry also identifies the hras(q61r) mutation in medullary thyroid cancer and may have a role in triaging genetic testing for men2. Am. J. Surg. Pathol2017;41;75–81. [PubMed: 27635947]
34. Marcadis AR, Valderrabano P, Ho ASet al.Interinstitutional variation in predictive value of the thyroseq v2 genomic classifier for cytologically indeterminate thyroid nodules. Surgery2019;165;17–24. [PubMed: 30360906]
35. Apel RL, Ezzat S, Bapat BV, Pan N, LiVolsi VA, Asa SL. Clonality of thyroid nodules in sporadic goiter. Diagn Mol Pathol1995;4;113–121. [PubMed: 7551291]
36. Jeong SH, Hong HS, Lee EH, Kwak JJ, Lee JY. Analysis of ras mutation in thyroid nodular hyperplasia and follicular neoplasm in a korean population. Endocrinol Diabetes Metab2018;1;e00040. [PubMed: 30815568]
37. Ohori NP, Landau MS, Carty SEet al.Benign call rate and molecular test result distribution of thyroseq v3. Cancer Cytopathol2019;127;161–168. [PubMed: 30561907]
38. Lloyd RV, Asa SL, LiVolsi VAet al.The evolving diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (niftp). Hum Pathol2018;74;1–4. [PubMed: 29339178]
39. Guo Z, Lloyd RV. Use of monoclonal antibodies to detect specific mutations in formalin-fixed, paraffin-embedded tissue sections. Hum Pathol2016;53;168–177. [PubMed: 27083401]
40. Castellano E, Santos E. Functional specificity of ras isoforms: So similar but so different. Genes Cancer2011;2;216–231. [PubMed: 21779495]
41. Nonaka D, Tang Y, Chiriboga L, Rivera M, Ghossein R. Diagnostic utility of thyroid transcription factors pax8 and ttf-2 (foxe1) in thyroid epithelial neoplasms. Mod Pathol2008;21;192–200. [PubMed: 18084247]
42. Wang JR, Zafereo ME, Dadu Ret al.Complete surgical resection following neoadjuvant dabrafenib plus trametinib in braf(v600e)-mutated anaplastic thyroid carcinoma. Thyroid2019;29;1036–1043. [PubMed: 31319771]
43. Yoshihara K, Wang Q, Torres-Garcia Wet al.The landscape and therapeutic relevance of cancer-associated transcript fusions. Oncogene2015;34;4845–4854. [PubMed: 25500544]

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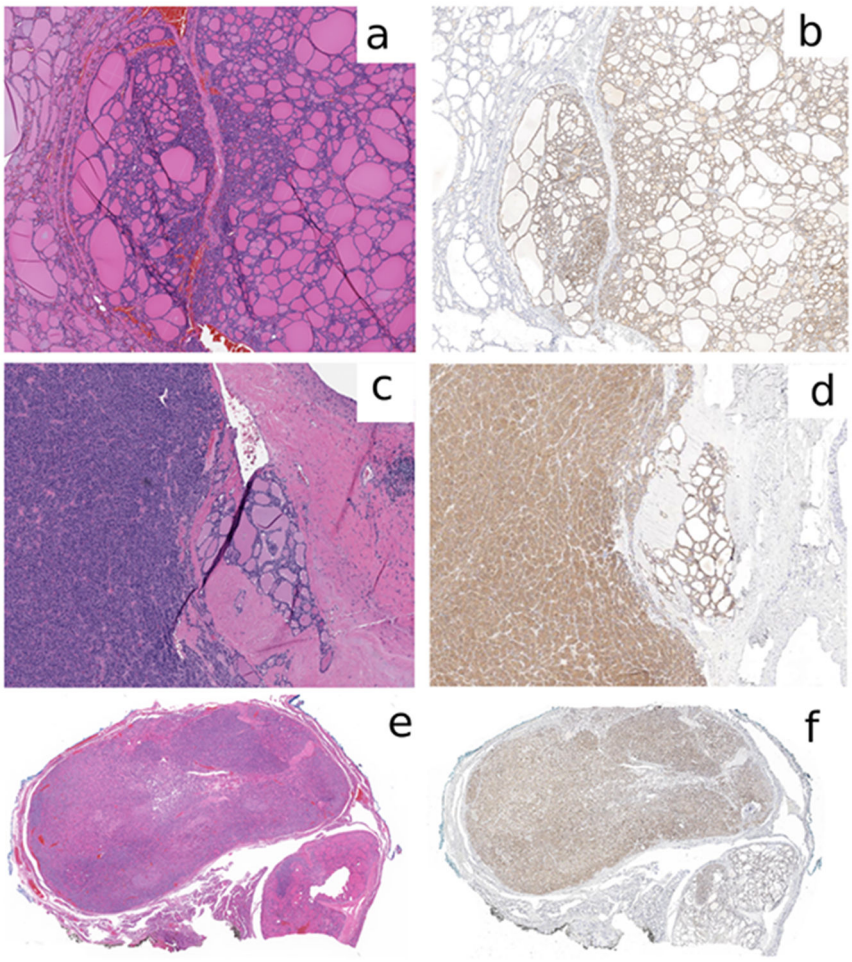

Figure 1. NRAS Q61R highlighting capsular invasion.

NRAS Q61R facilitates the identification of lesional buds qualifying for capsular invasion (image 1a, H&E, 2x and image 1b, NRAS Q61R, 2x), particularly in cases where the satellite nodule exhibits contrasting histologic features with those of the main nodule (image 1c, H&E, 2x and image 1d, , NRAS Q61R, 2x) or when it is situated further away from the capsule and lacks the classic mushroom shape (image 1e, H&E, 2x and image 1f, NRAS Q61R, 2x).

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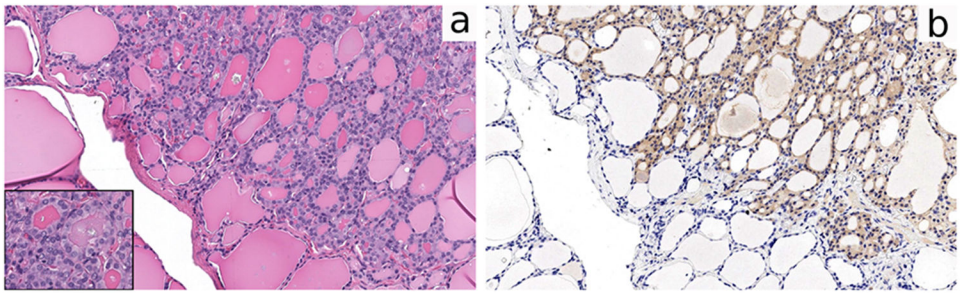

Figure 2. NRAS Q61R highlighting infiltration.

Image 2a (H&E, 20x) shows the border of a papillary thyroid carcinoma (PTC), well-demarcated follicular variant, with PTC nuclei depicted in the inset. Though the neoplastic and non-neoplastic follicles are in close proximity, definite infiltration could not be demonstrated based on H&E examination only. NRAS Q61R (image 2b, NRAS Q61R, 20x) demonstrates the limited infiltration of non-neoplastic NRAS Q61R-negative follicles by NRAS-positive neoplastic follicles.

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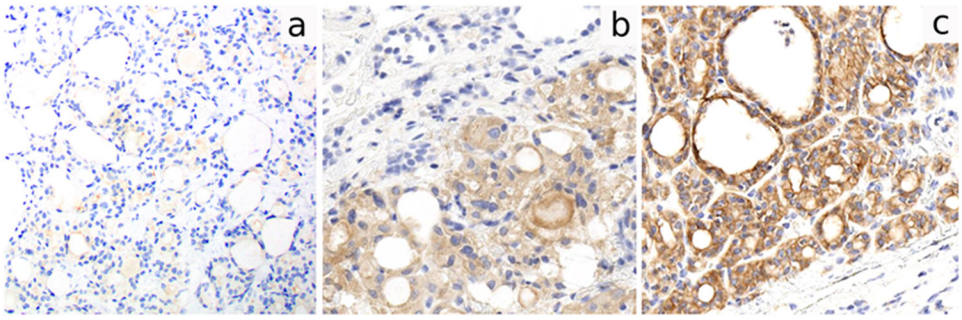

Figure 3. NRAS Q61R IHC interpretation.

Image 3a (NRAS Q61R, 20x) demonstrates weak (1+) staining intensity while images 3b and 3c (NRAS Q61R, 20x) demonstrate moderate (2+) and strong (3+) staining, respectively. Only moderate and strong intensities were considered as positive immunoreactivity.

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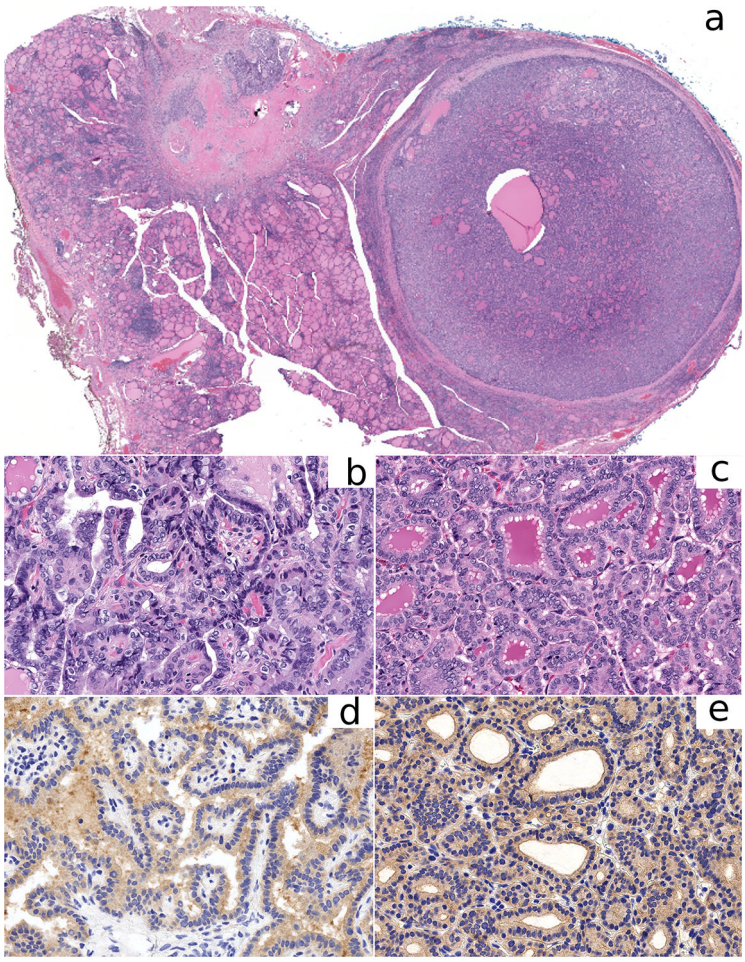

Figure 4. NRAS Q61R demonstrating multifocal carcinoma.

A different staining pattern for NRAS Q61R strongly argues in favor of separate neoplasms with dissimilar driver mutations. In this example (Image 4a, H&E, 4x) two adjacent foci of tumor consisting of an infiltrative classic papillary thyroid carcinoma (left side of image 4a with high power depicted in image 4b, H&E, 20x) and an encapsulated follicular variant papillary thyroid carcinoma (Right side of image 4a with high power depicted in image 4c, H&E, 20x) are seen. Immunostaining of the former for BRAF V600E (image 4d, BRAF V600E, 20x) and the latter for NRAS Q61R (image 4e, NRAS Q61R, 20x) confirms that these are two separate tumor cell lines.

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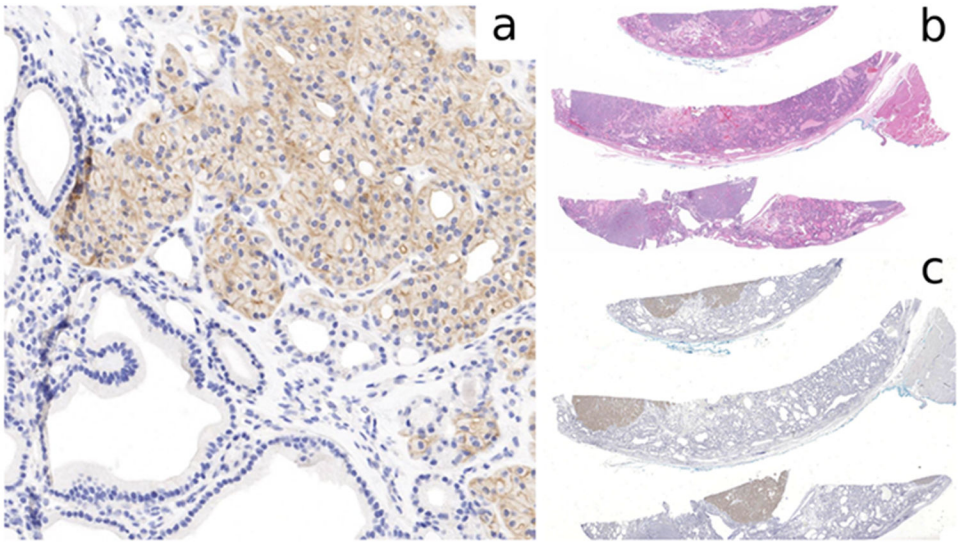

Figure 5. NRAS Q61R delineating the neoplastic process.

Image 5a (NRAS Q61R, 20x) depicts a papillary thyroid microcarcinoma, well-demarcated follicular variant with minimal infiltration, deceptively arising within a larger non-neoplastic encapsulated lesion (image 5b, H&E, 2x). Concern of whether the entire nodule represented a papillary thyroid carcinoma showing a spectrum of nuclear changes was resolved based on the demonstration of NRAS staining limited to the microcarcinoma (image 5c, NRAS Q61R, 2x).

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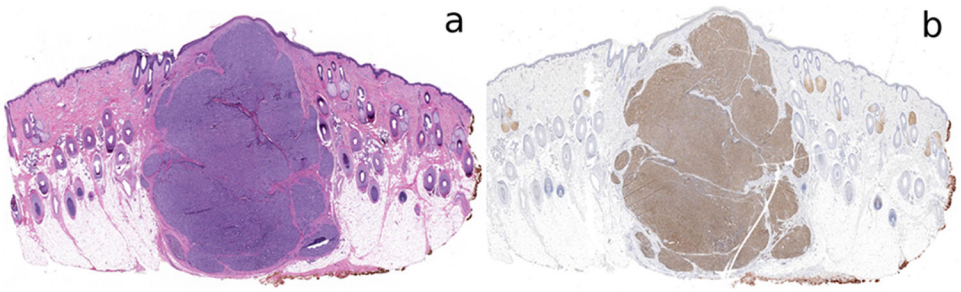

Figure 6. NRAS Q61R matching a metastatic lesion with a thyroid primary.

Image 6a (H&E, 2x) shows a subcutaneous metastatic carcinoma involving the scalp. Immunoreactivity for NRAS Q61R (image 6b, 2x) alongside a panel of other primary thyroid markers helped confirm this lesion to be a metastatic *RAS*-mutated poorly differentiated thyroid carcinoma.

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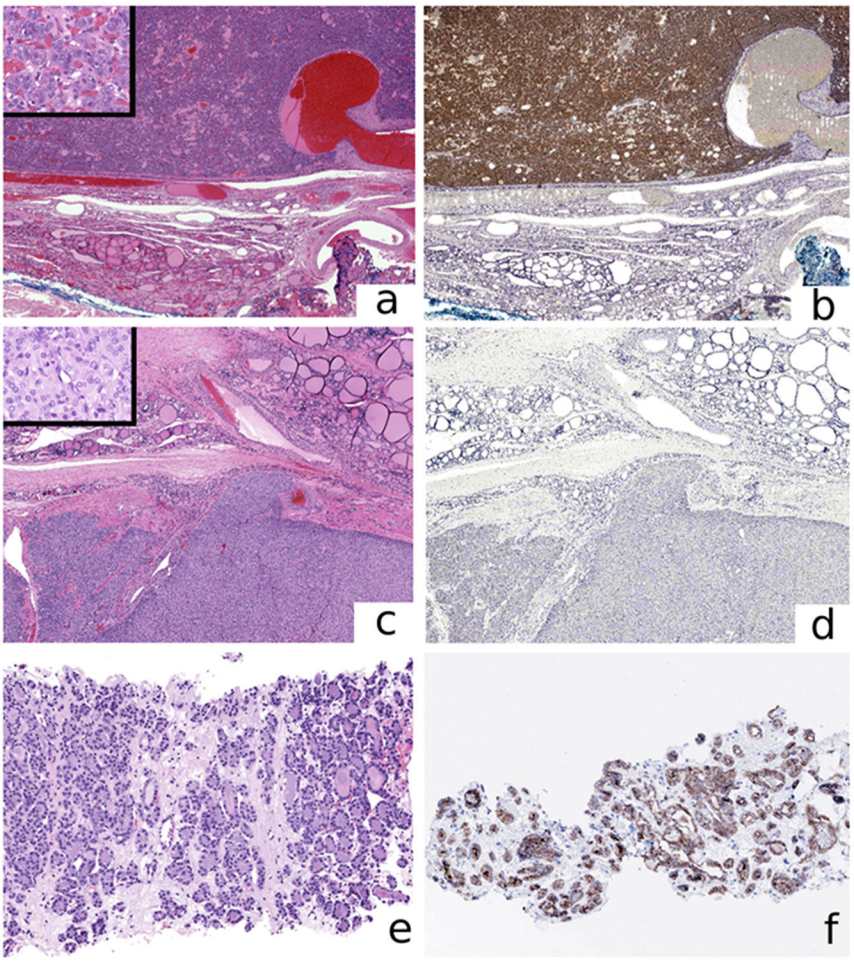

Figure 7. NRAS Q61R identifying a metastatic primary amongst multifocal thyroid carcinomas.

Image 7a (H&E, 4x) depicts a papillary thyroid carcinoma, encapsulated follicular variant that is immunoreactive for NRAS Q61R (image 7b, NRAS Q61R, 4x), while image 7c (H&E, 4x) shows a papillary thyroid carcinoma, encapsulated follicular variant that is NRAS Q61R negative (image 7d, NRAS Q61R, 4x). These two synchronous carcinomas were seen in the total thyroidectomy specimen of a patient who presented with a renal mass found to be a metastatic thyroid carcinoma (image 7e, H&E, 10x). Positive NRAS Q61R immunohistochemistry in the renal metastasis (image 7f, NRAS Q61R, 10x) helped identify the former tumor (images 7a and 7b) as the primary metastatic lesion and therefore as the correct nodule to genotype.

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Table 1-

Rates of NRAS Q61R IHC and RAS mutations positivity according to histopathologic diagnosis

	NRAS Q61R IHC	*RAS* Q61R genotyping	*NRAS* Q61R genotyping
Diagnosis	**Positive cases/ cases tested (%)**	**Mutated cases/ cases tested (%)**	**Mutated cases/ cases tested (%)**
Non-neoplastic	2/11 (18.2%)	-	-
Nodular hyperplasia	2/10 (20%)	-	-
Thyroid remnant	0/1	-	-
Benign	2/9 (22.2%)	0/3	0/3
Follicular adenoma	1/5 (20.0%)	0/2	0/2
Hurthle cell adenoma	1/3 (33.3%)	0/1	0/1
Hyalinizing trabecular tumor	0/1	-	-
Noninvasive follicular thyroid neoplasm with papillary-like nuclear features	28/67 (41.8%)	11/25 (44.0%)	6/25 (24.0%)
Malignant	50/195 (25.6%)	21/56 (37.5%)	14/56 (25.0%)
Primary	47/183 (25.7%)	20/50 (40.0%)	13/50 (26.0%)
Anaplastic carcinoma	2/6 (33.3%)	2/5 (40.0%)	1/5 (20.0%)
Poorly differentiated carcinoma	4/16 (25.0%)	3/9 (33.3%)	1/9 (11.1%)
Papillary carcinoma	40/148 (27.0%)	15/34 (44.1%)	11/34 (32.3%)
Follicular variant	37/77 (48.0%)	15/28 (54.0%)	11/28 (39.3%)
Infiltrative	1/4 (25.0%)	-	-
Encapsulated/well-demarcated	36/73 (49.3%)	15/28 (53.5%)	11/28 (39%)
Encapsulated with CI and/or VI	29/61 (47.5%)	14/26 (53.8%)	10/26 (38.4%)
Well-demarcated with MI	7/12 (58.3%)	1/2 (50.0%)	1/2 (50.0%)
Classic	2/61 (3.3%)	0/5	0/5
Tall cell variant	0/5	-	-
Solid variant	1/5 (20.0%)	0/1	0/1
Follicular carcinoma	1/7 (14.3%)	0/1	0/1
Hurthle cell carcinoma	0/6	0/1	0/1
Metastasis	3/12 (25.0%)	1/6 (16.7%)	1/6 (16.7%)
Anaplastic carcinoma	0/4	0/3	0/3
Poorly differentiated carcinoma	3/6 (50.0%)	1/3 (33.3%)	1/3 (33.3%)
Hurthle cell carcinoma	0/1	-	-
Papillary carcinoma	0/1	-	-

Abbreviations: capsular invasion (CI); vascular invasion (VI); minimal *infiltration (MI)*

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Table 2-

Association of NRAS Q61R IHC expression according to RAS genotype

		NRAS Q61R IHC		Total
		Negative, n (% of total tested)	**Positive, n (% of total tested**	
***RAS* Q61R genotyping**	***RAS* Q61R mutation not detected**	48 (57.1%)	4 (4.8%)	52
	***RAS* Q61R mutation detected**	3 (3.6%)	29 (34.5%)	32
	***NRAS* Q61R**	2	18	
	***HRAS* Q61R**	1	7	
	***KRAS* Q61R**	0	4	
Total		51	33	84