Immunohistochemical Detection of NRASQ61R Mutation in Diverse Tumor Types

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ABSTRACT

Objectives: Testing for NRAS mutation at codon Q61 is of therapeutic, prognostic, and diagnostic importance for metastatic melanoma, thyroid carcinoma, and colorectal carcinoma. Immunohistochemistry for NRASQ61R, the most common NRAS mutation, offers several practical advantages over current molecular diagnostic techniques.

Methods: We investigated the sensitivity and specificity of NRASQ61R in a series of 149 tumors with known NRAS genotype (72 malignant melanomas, 13 melanocytic nevi, 28 thyroid carcinomas, 25 gastrointestinal carcinomas, and 11 other malignancies).

Results: Thirty-five cases harbored the NRASQ61R mutation (19 malignant melanomas, one melanocytic nevus, 10 thyroid carcinomas, two gastrointestinal carcinomas, and three other malignancies). In this series, the concordance rate between immunohistochemistry and mutational analyses was 100%. The sensitivity and specificity were 100% and 100%, respectively. However, lower staining intensity was observed for thyroid carcinomas in comparison to melanomas and other tumors.

Conclusions: Our studies confirmed that immunohistochemistry provides excellent sensitivity and specificity for detecting the NRASQ61R mutation in a variety of tumor types in a clinical setting.

Upon completion of this activity you will be able to:
• recognize that NRAS mutation is commonly seen in a variety of malignancies, including malignant melanoma, thyroid carcinoma, colorectal carcinoma, and others.
• recognize that immunohistochemistry is a cost-effective means to screen for NRASQ61R mutation, which can have therapeutic, prognostic, and diagnostic implications.

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Testing for NRAS mutation at codon Q61 is of therapeutic, prognostic, and diagnostic importance for metastatic melanoma, thyroid carcinoma, and colorectal carcinoma. In melanomas, acquired resistance to BRAF inhibition can be mediated by NRAS mutations, most often Q61R and Q61K.1 In a recent phase II clinical trial, the MEK inhibitor MEK162 has shown clinical benefit for NRAS mutant melanoma, most being NRASQ61R.2 In addition, a combination of CDK4/6 (cyclin-dependent kinase 4) inhibitor, LEE011, with MEK162 is currently being evaluated in a phase Ib/II clinical trial for NRAS-mutated melanoma.3 On the contrary, in metastatic colorectal cancer, NRAS mutations are associated with poor prognosis and lack of response to anti–epidermal growth factor inhibitors.
receptors (EGFRs). Similarly, recent meta-analysis showed that KRAS, NRAS, BRAF, and PIK3CA mutations predict resistance to anti-EGFR therapies. NRAS codon 61 mutation has recently been reported to be a predictive marker for distant metastasis in follicular thyroid carcinoma. It also might serve as a diagnostic marker in thyroid pathology.

NRASQ61R is the most prevalent activating mutation in NRAS, and immunohistochemistry for this variant, made recently possible by the availability of a specific antibody, offers several practical advantages over current molecular diagnostic techniques, including more rapid analysis, less expense, greater tissue conservation, and wider laboratory availability. Furthermore, immunohistochemistry may allow for the detection of NRASQ61R in small tumor foci and for the assessment of intratumoral heterogeneity for the NRAS genotype, observed in previous studies, without the need for the laborious and costly microdissection required for a molecular-based approach. Although a monoclonal antibody has recently been shown to be highly sensitive and specific for the immunohistochemical detection of NRASQ61R in malignant melanomas with this specific mutation, a practical comparison of its performance in the clinical setting for various tumor types has not been done. We aim to evaluate its sensitivity and specificity in the detection of NRASQ61R mutation in melanomas, thyroid carcinomas, colorectal carcinomas, and other tumors.

Materials and Methods

Archival, formalin-fixed, and paraffin-embedded materials of tumors submitted for SNaPshot (Applied Biosystems, Waltham, MA) genotyping assay at the Molecular Diagnostic Laboratory, Massachusetts General Hospital, Boston, Massachusetts, between August 2010 and December 2014 were retrieved from the pathology files. Whenever possible, the same tumor block on which molecular testing performed was selected for immunohistochemical studies. Thirteen cases of melanocytic nevi with known NRAS genotype have been published previously.

Mutational Analysis

The SNaPshot genotyping assay and a recently developed targeted next-generation sequencing approach have been used for mutation detection in formalin-fixed, paraffin-embedded tumor tissue.

Immunohistochemistry

Immunohistochemical studies were performed on 5-µm-thick sections of formalin-fixed, paraffin-embedded tissue in a Bond 3 automated immunostainer (Leica Microsystems, Bannockburn, IL) and primary antibodies against NRASQ61R mutation (clone: SP174, 1:50, Spring Bioscience, Pleasanton, CA). Appropriate positive and negative controls were included. Positive staining was characterized by diffuse cytoplasmic with some membranous staining of the tumor cells. A malignant melanoma with a known NRASQ61R mutation was used as positive control. Diffuse staining in most of the tumor cells was noted in the positive cases. The intensity of staining was graded as strong (3+), moderate (2+), or weak (1+). No significant background staining was seen in the negative cases.

Results

The study included a total of 159 specimens. Of the 149 specimens with known NRAS genotype, there were 72 cases of malignant melanoma (20 primary and 52 metastatic melanomas), 13 melanocytic nevi, 25 cases of gastrointestinal carcinomas (14 primary and 11 metastatic carcinomas), 28 cases of thyroid carcinomas (23 primary and five metastatic carcinomas), six lung carcinomas (three primary, three metastatic), and five miscellaneous tumors (one transformed mycosis fungoides, one plasmacytoma, one plasmacytoid dendritic cell neoplasm, two ovarian serous carcinomas). In addition, archival materials without known NRAS genotype of corresponding primary or metastatic tumors from 10 patients were included to assess intertumoral homogeneity.

Concordance Between Immunohistochemistry and Mutational Analyses

The overall concordance rate between protein expression and mutation was 100% (Table II). The sensitivity and specificity were 100% and 100%, respectively.

Nineteen malignant melanomas with NRASQ61R mutation were positive (15 with 3+ and four with 2+), while the remaining 53 melanomas (10 with Q61K, four with Q61L, one with Q61H, one with G12D, and 37 wild type) were all negative. One congenital nevus with NRASQ61R mutation was 2+, positive, while the remaining 12 nevi (one with Q61K, two with G13D, and nine wild type) were negative. Two metastatic colonic adenocarcinomas harboring the NRASQ61R mutation were both positive (3+), and the remaining 23 gastrointestinal carcinomas (two with Q61K, one with G12A, one with G12C, two with G12D, one with G13D, and 16 wild type) were negative.

Ten thyroid carcinomas with NRASQ61R mutation were all positive (one with 2+ and nine with 1+). One metastatic lung squamous cell carcinoma, and one metastatic ovarian serous carcinoma mutant with NRASQ61R were all 2+, 2+, and 3+ positive, respectively.
Concordance Between Primary and Metastasis of the Same Patient

Additional archival materials of primary or metastatic tumors were available in 10 patients with specimens with known NRAS genotype. The mutational analyses were not done on these corresponding samples. With the exception of one case, there was high concordance of NRASQ61R expression in both tumor specimens from the same patient [Table 3].

Discussion

KRAS, BRAF, or NRAS mutations can activate the RAS-RAF-MAPK pathway, which is important for proliferation. Activating KRAS, HRAS, or NRAS mutations at codons 12, 13, or 61 can be seen in one-third of cancers. RAS proteins function as guanosine triphosphatase (GTPase) switches. Mutations at codons 12 and 13 render RAS proteins insensitive to GTPase-activating proteins, and mutations at codon Q61 block the return of RAS to an inactive guanosine diphosphate–bound state. Similar to BRAF mutations, NRAS mutations are driver mutations on which a tumor is dependent for proliferation. Approximately 15% to 25% of metastatic melanomas possess NRAS (neuroblastoma RAS viral oncogene homolog) mutations, and they are localized to codon 61 in 82%, codon 12 in 13%, and codon 13 in 5% of cases. Preference for codon 61 mutations is also observed in thyroid carcinomas. RAS mutations are the second most common genetic changes in thyroid tumors seen in 10% to 20% of papillary thyroid carcinomas and 40% to 50% of follicular thyroid carcinomas. Mutations of codon 61 can be seen in 67% of NRAS-mutated follicular
Strong and diffuse cytoplasmic expression of NRASQ61R is seen in representative metastatic melanoma in soft tissue (A, ×200; B, ×200) and metastatic colonic adenocarcinoma to bone (C, ×100, D, ×100), while only weak cytoplasmic expression is seen in a follicular thyroid carcinoma (E, ×100, inset ×400, F, ×400).
NRASQ61R Staining in Primary and Corresponding Metastasis From the Same Patienta

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With NRAS Mutational Analyses</th>
<th>NRASQ61R Staining</th>
<th>Other Tumor in Same Patient Without NRAS Mutational Analyses</th>
<th>NRASQ61R Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma with NRASQ61R mutation</td>
<td>Primary</td>
<td>Positive</td>
<td>Primary</td>
<td>Positive</td>
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<tr>
<td>Metastasis</td>
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<td>Metastasis</td>
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<td>Primary</td>
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<tr>
<td>Melanoma with NRASQ61K mutation</td>
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<td>Negative</td>
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<td>Recurrence</td>
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<td>Thyroid carcinoma with NRASQ61R mutation</td>
<td>Metastasis</td>
<td>Positive</td>
<td>Primary</td>
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aThe cases listed in the second column are with known NRAS genotyping, while those listed in fourth column did not have NRAS genotyping done.

caryomas. NRAS-mutated thyroid cancers are often a follicular variant of papillary thyroid carcinoma.8,18

In this study, we did not optimize an individual protocol for each tumor type but rather used one uniform protocol. We observed less staining intensity for thyroid carcinomas in comparison to melanoma cases and gastrointestinal and lung carcinomas. It is unclear why there was different staining intensity noted in different tumor types in our study. It could be due to the variability in protein expression in different tumor types, as noted for VE1 antibody, which better detects BRAFV600E mutation in melanoma and papillary thyroid carcinoma than colorectal carcinoma.20 We showed here that the immunohistochemistry analysis for NRASQ61R is a technically less challenging, more rapid, and cost-effective alternative to molecular testing.

Mutations at codon 61 and codon 12 are commonly seen in melanoma, thyroid cancer, and colorectal carcinoma. NRASQ61R is the most common NRAS mutation found in melanoma seen in approximately 35% of NRAS-mutated melanomas.21 It is also found in follicular carcinoma and follicular variant of papillary thyroid carcinoma.18 Thirty-six cases with seven different NRAS variant mutations other than Q61R (Q61L, Q61K, Q61H, G12A, G12C, G12D, and G13D) were included in this study, and all cases were negative for NRASQ61R immunostain. NRASQ61K is a commonly recurring NRAS mutation seen in approximately 34% of NRAS-mutated melanomas and in hereditary melanoma.21 Both mutations can be seen in congenital melanocytic nevi.22 The Q61L and Q61H mutations are seen in approximately 8% and 2% of NRAS-mutated melanomas, respectively. The G12D mutation is the third most common NRAS mutation, which is a recurring mutation in colorectal carcinoma23 and melanoma.17

High concordance between matched primary and metastatic melanomas has been reported for BRAFV600E antibody.24 Similarly, intertumoral homogeneity for melanoma and thyroid carcinoma was noted for the NRASQ61R antibody in this study (Table 3). Although future confirmation with additional cases will be valuable, there appears to be no need for additional biopsy provided there is a specimen with adequate tumor cellularity. These findings are supportive of the fact that driver mutations, including oncogenic NRAS and BRAF mutations, are typically somatically preserved during progression.

As the role of targeted therapy and therapy-guiding NRAS mutational analysis expands to include a spectrum of malignancies beyond melanoma, NRASQ61R immunostain offers timely clinical diagnosis of NRASQ61R expression in malignancies of various sites and tumor types. Our results confirmed that a monoclonal antibody provides excellent sensitivity and specificity for detecting the NRASQ61R mutation in a variety of tumor types, for primary as well as metastatic tumors.

References


