KRAS and BRAF Mutation Spectrum In 1,035 FFPE Tumor Samples Submitted For Clinical Testing

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Introduction

Activating mutations in both the KRAS and BRAF genes are associated with poor prognosis and non-response to anti-EGFR therapies in patients with advanced cancers. Clinical guidelines have recommended that KRAS mutational status be determined in all patients with metastatic colorectal cancer that are being considered for anti-EGFR therapies. In this study, a pathologist reviewed an H&E slide of each tumor sample and determined the percent tumor nuclei and percent necrosis. Each specimen was macrodissected and DNA was extracted. DNA was then amplified with primers flanking KRAS exon 2 (codons 12 and 13), and KRAS exon 3 (codon 61), and BRAF exon 15. Using the ABI3730 and Mutation Surveyor software, mutations were scored as positive when evident in both the forward and reverse reactions. A total of the 1,035 clinical tumor specimens were submitted to our lab for KRAS and/or BRAF mutation analysis by direct sequencing or PCR analysis.

Results

KRAS and BRAF in α-EGFR Antibody Resistance

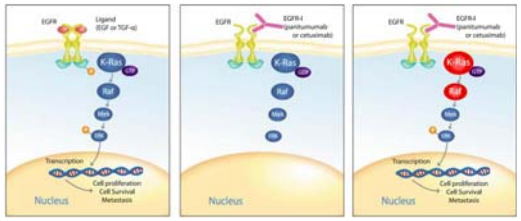


FIGURE 1: A Diagram of the EGFR-MAPK Signaling Pathway. (Left) Upon ligand binding the EGFR receptor dimerizes and is autophophorylated. Upon autophosphorylation the EGFR stimulates a guanonucleotide exchange factor to activate KRAS. Activation of KRAS leads to activation of downstream elements, including BRAF, to promote cell proliferation and survival. (Center) α-EGFR antibodies (cetuximab and panitumumab) compete with EGFR ligands for binding to the receptor. This competition inhibits KRAS activation causing reduced signaling for cell proliferation and survival. (Right) When either KRAS or BRAF are constitutively activated by mutation (shown in red) proliferation and survival signaling occurs even in the presence of α-EGFR antibodies. Figure adapted from Siddiqui et al., 2010.

Mutation Spectrum of Samples Tested for KRAS

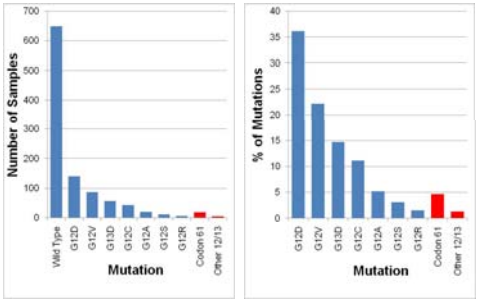


FIGURE 2: KRAS Mutations Observed in 1035 Clinical Samples Submitted for Testing. Tumor samples were sectioned and scored for areas with >50% tumor nuclei by a pathologist and macrodissected by a technologist. Isolated DNA was PCR amplified and sequenced for mutations in codons 12, 13 and 61. The most common mutation identified in our laboratory was p.G12D (c.35G>A) and was 36.2% of all mutations observed. Bars in red represent mutations that are not analyzed in a commercially available PCR kit that is used in our laboratory. The codon 61 and other codon 12 and 13 mutations (frequency in parenthesis) were p.Q61H (8), p.Q61K (5), p.Q61L (3), p.Q61R (2), p.G13R(2), G12L (1), p.G13dup (1), and G13C (1).

KRAS Mutations in Different Tumor Types

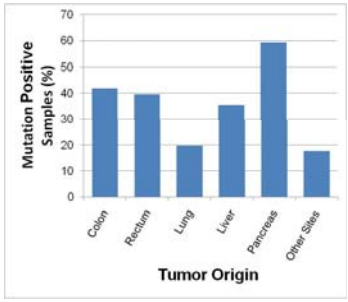


FIGURE 3: KRAS Mutations Observed in Tumors Arising From Different Tissues. Samples were classified based on the tissue of origin that was provided on the requisition form submitted with the sample. Consistent with previously reported results, ~40% colorectal cancer and ~60% of pancreatic cancer specimens were positive for mutations in KRAS. Notable "other sites" that were tested for KRAS mutations were skin(15), breast (13), stomach (9), cecum (8), esophagus (8), and neck (6).

Results

KRAS Mutation Spectrum for Colorectal Cancer

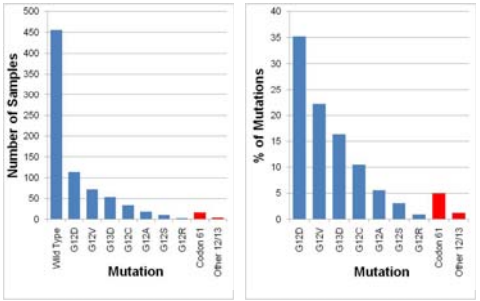


FIGURE 4: Variation In KRAS Mutations Observed in 780 Colorectal Samples Submitted for Testing. Tumor samples were sectioned and scored for areas with >50% tumor nuclei by a pathologist and macrodissected by a technologist. Isolated DNA was PCR amplified and sequenced for mutations in codons 12, 13 and 61. The most common mutation identified in our laboratory was p.G12D (c.35G>A) and was 35.2% of all mutations observed. Bars in red represent mutations that are not analyzed in a commercially available PCR kit that is used in the laboratory. The codon 61 and other codon 12 and 13 mutations (frequency in parenthesis) were p.Q61H (8), p.Q61K (5), p.Q61L (3), p.Q61R (2), p.G13R(2), G12L (1), p.G13dup (1), and G13C (1).

KRAS Mutations Identified by PCR Analysis

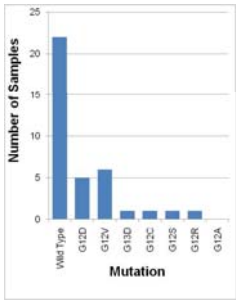
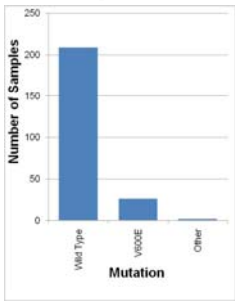
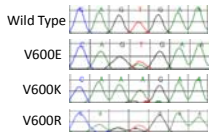


FIGURE 5: Variation In KRAS Mutations Observed in 37 Samples Tested By PCR. Tumor samples with 10-50% tumor were analyzed for KRAS mutations using a commercially available PCR based kit. This kit tested for the seven most common mutations listed on the chart above. Unlike results obtained from mutational analysis performed by sequencing, the most common mutation identified was p.G12V (c.35G>T). Overall, ~40% of samples were positive for KRAS mutations as determined by PCR analysis.

Mutation Spectrum of Samples Tested for BRAF





Tissue	Positive	Total	% Positive
Colon	18	178	10.1%
Skin	8	13	61.5%
Rectum	0	14	0%
Lung	1	7	14.3%
Thyroid	1	4	25%
Pancreas	0	6	0%
Other	0	15	0%
Total	28	237	11.8%

FIGURE 6: Variation in BRAF Mutations Observed in 237 Clinical Samples Submitted for Testing. Tumor samples were sectioned and scored for areas with >50% tumor nuclei by a pathologist and macrodissected by a technologist. Isolated DNA was PCR amplified and sequenced for mutations in codon 600. (Left) The most common mutation identified in laboratory was p.V600E (c.1799T>A). Other variants that were found were p.V600K and p.V600R. (Top Right) Chromatograms depicting wild type, p.V600E, p.V600R, and pV600K mutations in exon 15 of BRAF are shown. (Bottom Right) A chart displaying the mutation rates of tumors arising from several different tissues is shown. Other tissues tested were ovary (3), liver (3), stomach (2), bowel (2), lymph node (2), duodenum (1), jejunum (1), Ewing sarcoma (1), and other intestine (1).

Conclusions

- ~40% of colorectal samples were positive for KRAS mutations
- The most common mutation observed in KRAS was p.G12D
- 4.6% of all mutations observed in KRAS occurred in codon 61
- Testing for the 7 most common KRAS variants would have a clinical sensitivity of 94%.
- V600E mutations in BRAF comprised ~93% of all mutations observed in exon 15
- Two rare variants were observed in exon 15 of BRAF (p.V600R and p.V600K) in skin samples
- ~10% of colon cancer samples and ~60% of skin samples were positive for BRAF

References:

Siddiqui, A.D. and B. Piperdi, KRAS mutation in colon cancer: a marker of resistance to EGFR-I therapy. Ann Surg Oncol, 2010. 17(4): p. 1168-76.