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The potential utility of re-mining results of somatic mutation testing: *KRAS* status in lung adenocarcinoma

Anna Biernacka [a], Peter D. Tsongalis [b], Jason D. Peterson [a], Francine B. de Abreu [a], Candice C. Black [a], Edward J. Gutmann [a], Xiaoying Liu [a], Laura J. Tafe [a], Christopher I. Amos [b,c], Gregory J. Tsongalis [a,b,*]

[a] *Department of Pathology and Laboratory Medicine, Dartmouth Hitchcock Medical Center and Norris Cotton Cancer Center, Lebanon, NH, USA;* [b] *the Geisel School of Medicine at Dartmouth, Hanover, NH, USA;* [c] *Department of Community and Family Medicine, Dartmouth Hitchcock Medical Center and Norris Cotton Cancer Center, Lebanon, NH, USA*

KRAS mutant non-small cell lung cancers (NSCLCs) vary in clinical outcome depending on which specific *KRAS* mutation is present. Shorter progression free survival has been associated with *KRAS* variants G12C and G12V. Cell lines with these variants depend to a greater extent on the *RAS/RAF/MEK/ERK* signaling pathway and become more susceptible to *MEK* inhibition. Because different *KRAS* mutations may lead to altered drug sensitivity, we aimed to determine specific *KRAS* mutation status in a NSCLC patient cohort at our institution. A total of 502 NSCLC samples were screened for somatic mutations using the 50 gene AmpliSeq™ Cancer Hotspot Panel v2 (CHPv2). However only samples positive for variants in the *KRAS* gene were included in this study. Variants identified in the *KRAS* genes were curated using publicly available databases. The overall mutation rate in the *KRAS* gene was 32.7% (164/502). The most common *KRAS* mutations were G12C (41%), G12V (19%), and G12D (14%) along with less frequent variants. After re-mining our sequencing data, we found that more than a half of our *KRAS* mutant NSCLC patients could potentially benefit from the addition of a *MEK* inhibitor such as selumetinib to standard chemotherapeutic agents. Due to mutated *KRAS*, these patients will likely fail traditional anti-*EGFR* therapies but be eligible for newer combination therapies.

Keywords Somatic mutation, KRAS, lung cancer, next generation sequencing, data mining

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Introduction

Personalized medicine efforts with respect to oncology are heavily dependent on somatic mutation analysis of tumor cells that identifies gene mutations associated with response, positive or negative, to novel targeted therapies. Numerous molecular technologies are available for use in such testing and in many instances a combination of techniques and/or assays is used to identify a tumor mutation profile that can then be used in the design of a management strategy for a particular patient.

As new therapeutics were introduced after clinical trials and FDA approval, the need for companion diagnostics far outpaced the single gene, single mutation types of assays that many labs were offering. Throughput of traditional multiplexed assays and Sanger sequencing were also overtaken by increasing demand for more information about additional genes representing a variety of targeted pathways. Many laboratories validated SNaPshot assays which increased numbers of detectable mutations in a single test as well as next generation sequencing (NGS) (1). Our lab initially introduced NGS for clinical testing by validating the Ion Torrent AmpliSeq Hotspot Cancer Panel, a pancancer 50 gene hotspot test that is routinely run on primary and metastatic colorectal cancers, and

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* Corresponding author.
E-mail address: Gregory.j.tsongalis@hitchcock.org

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metastatic gliomas, melanomas, lung adenocarcinomas, and triple negative breast cancers (2).

The molecular reclassification of non-small-cell lung cancer (NSCLC) has changed the diagnostic and therapeutic approach and improved outcome for patients with EGFR mutations and ALK rearrangements as oncogenic drivers (3,4). Despite these advances, the largest known genetically defined subset of NSCLC, harboring mutation in KRAS oncogene, remains an elusive therapeutic target (5). KRAS mutations, associated with adenocarcinoma histology and tobacco use, have been linked to worse survival and lack of response to cytotoxic chemotherapy as well as anti-EGFR therapy (3–5). As compounds designed to specifically target RAS proteins, such as farnesyl transferase inhibitors, showed little efficacy, efforts have shifted to inhibit downstream effector proteins in RAS-RAF-MEK-ERK (MAPK) or PI3K/AKT/mTOR signaling pathways with promising yet variable response rates in preclinical and clinical testing (6–8). The particular challenge to effectively target the KRAS-driven cancers has been recently attributed to a greater molecular heterogeneity in tumors with mutated KRAS compared with tumors with other known oncogenic drivers (3,4,7). It has been shown that different KRAS amino acid substitutions differ in their patterns of downstream signaling pathways, suggesting that specific KRAS alleles may account for at least part of this diversity (7). Recently, Janne et al. identified a subset of *KRAS* mutation positive lung adenocarcinomas that showed sensitivity to the combined therapy of MEK1/MEK2 inhibitor selumetinib and docetaxel, supporting the notion that the biology of KRAS mutations differ, and the efficacy of targeted therapies may be related to the specific KRAS mutation (9,10) This study prompted us to re-review our NGS data on more than 500 lung adenocarcinomas to identify the frequency of specific *KRAS* mutations and the number of patients who might benefit from such combination therapy.

Materials and methods

Samples and DNA extraction

Five hundred and two formalin-fixed, paraffin-embedded (FFPE) tissues from non-small cell lung cancer (NSCLC) patients were received at Dartmouth-Hitchcock Medical Center (DHMC) between May 2013 and September 2015 for somatic mutation screening. However only samples positive for variants in the *KRAS* gene were included in this retrospective study.

The FFPE sample types received at DHMC included mainly surgical (50) and cytology (114). All hematoxylin and eosin (H&E) stained slides from each patient were reviewed by an attending pathologist who determined the tumor area and the percentage of tumor cells present in the tissue section. The tumor cellularity ranged from 10 to 95% in both sample types (surgical and cytology).

DNA extraction was performed using the Gentra Pure Gene Kit (Qiagen) or the QiaCube (Qiagen; after August 2015), and quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) according to the manufacturer's recommendations. DNA quality was assessed using the KAPA hgDNA Quantification and QC Kit (KAPA Biosystems, Wilmington, MA).

Next generation sequencing (NGS)

As determined during validation, samples with (1) tumor cellularity below 10% and (2) DNA concentration below 1.7 ng/μL and DNA quality ratio below 0.4 were not screened for somatic variants. Approximately 10 ng of genomic DNA from each sample was used to create barcoded libraries using the Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHPv2) (ThermoFisher). The CHPv2 panel consists of hotspot regions for 50 oncogenes and tumor suppressor genes covering approximately 2800 Catalogue of Somatic Mutations in Cancer (COSMIC) mutations. Barcoded libraries were combined to a final concentration of 100 pM using the Ion Library Quantitation Kit (ThermoFisher), and a maximum of 10 samples were sequenced on an Ion 318 Chip v2 using the Ion PGM System (ThermoFisher).

For data analysis, read mapping, alignment of sequences to hg19 (Human Genome Version 19), variant calling, and coverage analysis were performed using the Torrent Suite software (v4.0.2). Variant annotation and prediction of functional significance were performed using Golden Helix SNP & Variation Suite (SVS) software (v8.2.1). Reported variants passed the minimum reporting thresholds established during validation which includes 500× coverage, 5% allelic frequency and strand bias of 0.40–0.59.

For NSCLC, variants identified in the *BRAF, EGFR*, *KRAS*, or *PIK3CA* genes were characterized as clinically actionable according to NCCN Clinical Practice Guidelines in Oncology and My Cancer Genome: Genetically Informed Cancer Medicine. Curation of the variants identified in the *KRAS* genes was performed using publicly available databases. All reports were reviewed by a genomic informaticist before being reviewed and curated by a genomic analyst.

Results

The overall mutation rate in the *KRAS* gene was 32.7% among the NSCLC cases studied (164/502). The most common *KRAS* mutations were G12C (41%) and G12V (19%) that are both considered *RAS/RAF/MEK/ERK* pathway dependent. Another common subtype, G12D (14%), is known to act more through *AKT* phosphorylation. The remaining mutations included G12A (6%), Q61H (5%), G13C (4%), and rarer subtypes: G12R, G61L, G12S (2% each), and G13D, G13S, G12F, L19F, G13P (1% each). The different types of KRAS mutations found within our population are presented in Figure 1.

Discussion

An unprecedented amount of molecular data is being generated by analyzing human tumors in the name of personalized medicine. While the technologies used are evolving at a fast pace with costs decreasing faster than Moore's Law could have predicted, proven clinical utility of the generated data is lagging, in part due to the complexity of human cancers. Nonetheless, the impetus to develop novel therapeutic strategies to improve the management of the individual cancer patient drives this testing.

Single gene mutations identified in a specific tumor type, such as *BRAF* V600E in melanoma, have relatively straightforward interpretations with respect to response to select *BRAF*

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