Mutation Profiling of Colorectal Cancer ctDNA using AmpliSeq CHP2 Cancer Panel

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Introduction

Analysis of ctdNA in CRC has potential applications in early detection, monitoring response to therapy, tracking resistance and assessment of minimal disease. Emergence of resistance mutations in KRAS, NEAD, BRAF, EGFR and other genes after treatment with anti-EGFR mAbs therapy can be monitored non-invasively by serial “Liquid Biopsy” to allow detection of tumor recurrence months earlier than conventional imaging. To this end multiple approaches have been developed including BEAMing, ddPCR and next generation sequencing. Here we use an amplification based target enrichment method, AmpliSeq v2 (CHP2), to study a set of tumors/plasma pairs (n=12) collected from stage IV colon cancer patients to determine whether amplification based NGS can be used to profile mutations in ctdNA from CRC patients. A data analysis pipeline to detect novel ctdNA mutations was developed and trained using this dataset.

Methods

13 matched tumor and plasma samples were purchased from Indimut. Mutations were assessed using AmpliSeq v2 (CHP2) cancer panel, designed to survey 2800 mutations in 50 cancer-related genes. For mutation detection in tumor samples, we performed library prep in parallel for Ion Torrent and Illumina MiSeq using 10 ng DNA as shown in Figure 1. Plasma DNA sequencing was only performed using Ion Torrent PGM. Plasma DNA was extracted from 3 or 5 ml plasma using the QIAamp DSP circulating DNA kit and eluted in 20 ul buffer per ml plasma. The sample input used in the CH2 reactions was between 3.5 – 55 ng ctdNA. The background noise level of 13 library input positions was calculated from 10 healthy donor plasma samples. A custom data analysis program was developed to detect variants clearly different from inter-run and intra-run background noise.

Results

Determination of ctdNA concentration: The ctdNA samples can be divided into two groups based on the DNA concentration measured by Qubit (range of 0.10–13 ng/ul). 6 with <1 ng/ul and 7 with 1 ng/ul (Figure 2).

Number of mutations grouped by AF in tumor vs plasma: ctdNA mutations can be grouped into 3 levels: allele frequencies >5% (N=7), 1% (N=11) and between 0.1-10% (N=13).

ctDNA mutation pattern: Co-existing mutations in the same tumor tend to be detected simultaneously in the matched plasma at the same level of allele frequency (Figure 3). Two exceptions of this were that only driver mutations (KRAS G12D and PIK3CA E545G) were detected in two ctdNA samples (circled in red), while mutations in other genes (TP53, APC and SMAD4) were found only in the matched tumors, probably due to differential roles of these genes in cancer progression and metastasis.

Evaluating the mutation standard for ctdNA: As a mutation standard is needed for clinical assay, we evaluated Horizon Dx ctdDNA 0.1% reference standard, which is fragmented DNA from cell line mixes mimics the characteristics of plasma DNA. 10 ng of Horizon Dx ctdDNA 0.1% reference standard was analyzed using AmpliSeq CHP2 cancer panel. NGS results were compared to ddPCR results from HorizonDx. Most mutations except PIK3CA H1047R mutation were detected at the expected AF (Table 4). The drop-off of this mutation from the ddPCR standard is under investigation. However, our previous data in breast cancer showed that AmpliSeq v2 was able to detect PIK3CA H1047R mutation at 0.06 AF. Seven mutations were not tested by ddPCR but detected by AmpliSeq v2. These mutations determined to be true because they were reported by Cosmic mutation database in the cell line used for preparing the standard.

Conclusions

- AmpliSeq CHP2 cancer panel achieved 0.06-0.2% allele frequency sensitivity and 85% (11/13) tumor/plasma concordance, which are comparable with previous reports using alternative NGS panels.
- We also demonstrated the efficiency of target enrichment by AmpliSeq based multiplex PCR, which can detect as low as several mutant copies from wild type background.

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