Introduction

Targeted therapies in many cancers have allowed unprecedented progress in the treatment of disease. However, routine implementation of genomic testing is limited due to: 1) difficulties in detection of mutational loads below 5%, 2) limited amounts of sample (pg-ng range) per biological specimen, 3) diagnostic turnaround time and workflow, and 4) cost. KRAS is mutated in approximately 40% of colorectal cancers, and KRAS mutations are predictive of a negative response to eGFR therapy. To optimize therapy strategies for personalized care, it is therefore critical to rapidly screen patient samples for the presence of multiple KRAS mutations. We have developed a multiplexing strategy to screen clinically-actionable KRAS mutations using digital PCR. No pre-amplification step is required. This sensitive and inexpensive method reduces the risk of contamination and can be easily implemented in molecular diagnostic laboratories for rapid, routine screening of cancer patients.

Materials and Methods

- KRAS patient plasma samples (5 female, 6 male, average age 52 years, 52 draws) and 12 normal plasma (12 female) samples were purchased (Converseable, Norms, Ac) and (Prentivito, Surlyple, Lk) respectively. Free mCRC tissue samples were classified as KRAS mutation positive by the vendor, but not tested as plasma. Additional samples (Fig: J) were provided by the Janka lab at MDACC. Samples were prepared using standard protocols (Keatinge DNA).
- ddPCR was performed on – 0.75 uL per sample well using either the KRAS Screening Multiplex for ddPCR validated PrimePCR ddPCR mutation assays or one of seven individual KRAS mutations (G12D, G12V, G12S, G12C, G12R, G12S, Bio-Rad Laboratories)
- Positive mutation references were from Horizon Diagnostics, and negative controls were wild-type only from Promega (female gDNA).

Figure 1: Multiplexed single-well detection of 7 actionable KRAS mutations

Figure 2: Cell-free plasma samples yield highly variable amounts of amplifiable DNA

Figure 3: KRAS Screening Multiplex assay on cell line DNA, FFPE, and cfDNA from MD Anderson Cancer Center patients

Figure 4: Multiplex detection of KRAS mutations in 24 patient samples from cell-free plasma DNA

Figure 5: ddPCR enables visualization of PCR inhibition from FFPE and cell-free plasma DNA samples

Conclusions

- Droplet digital PCR is a inexpensive method to quantify, absolutely, minimal amounts of FFPE and cfDNA, both for quantification and mutation detection.
- The amplifiable amount of cfDNA is significantly different between normal and cancer patient samples, and between KRAS mutant and KRAS wild type patient samples.
- We have demonstrated sensitive and precise detection down to 0.25% for multiple actionable KRAS mutations in cfDNA plasma samples from colorectal cancer patients.
- Droplet digital PCR provides a simple and robust workflow to screen a large volume of patient samples in a minimal amount of time.
- 1D & 2D plots of droplets enables rapid identification of PCR inhibition, either by poor assay design, sample inhibitors, poorly optimized conditions, or template degradation.

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