Ultrasensitive Detection of Early-Stage Cancer Using ctDNA Sequencing with UMIs

Ding, Elizabeth (School: Lexington High School)

Circulating tumor DNA in blood holds promise as a cancer-specific biomarker. However, detection of ultra-low mutation allelic frequency (MAF) of ctDNA at early stages of cancer is infeasible by conventional next generation sequencing (NGS). Using duplex sequencing with unique molecular identifiers (UMIs) and custom-designed probes, we tested the hypothesis that ctDNA duplex sequencing with UMIs will be able to detect ultra-low MAF of ctDNA from early-stage cancers. We designed a 128-gene panel that contains probes targeted to clinical relevant genome variations of lung, gastric and esophageal cancers and validated with reference DNA and controls using ctDNA duplex sequencing with UMIs. A data analysis pipeline was implemented with improved algorithms for variant calling, bTMB calculation, and tumor classification. Pearson's correlation coefficient and t-test were used for statistical analysis. We designed and validated a ctDNA duplex sequencing with UMIs assay that enables simultaneous detection of 128 clinically relevant genes with SNPs, indels, amplifications, and fusions in a single blood test. Compared conventional ctDNA NGS, our assay achieved high sensitivity (over 75%) and specificity (over 96%) with LOD at 0.1% MAF for stage I cancers with sequencing depth at 30,000x. Results also show significant concordance of MAF between DNA from tumor tissues and plasma ctDNA. In this study, a novel ultrasensitive assay was designed and tested for accurate detection of MAF at 0.1% from plasma ctDNA of multiple tumors. Results from initial testing show its promising clinical applications for early cancer detection as a liquid biopsy.

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