Cell-free DNA (cfDNA) Fragment Length Patterns of Tumor- and Blood-derived Variants in Participants With and Without Cancer

Earl Hubbell, PhD; Tara Maddala, PhD; Oliver Venn, DPhil; Eric Scott, PhD; Susan Tang, MS; Archana Shenoy, PhD; Alex Aravanis, MD, PhD
GRAL, Inc., Menlo Park, CA

BACKGROUND

Previous studies of isolated tumor or single cancer samples have indicated that the fragment lengths of plasma-derived cfDNA differ from their tissue of origin. However, it is currently unknown whether cfDNA fragment lengths vary significantly among individuals with the same tumor type. This study aimed to address this question by analyzing a large number of plasma cfDNA samples from a diverse set of cancer patients.

METHODS

Sample Processing

Plasma samples (N=1406) were evaluated from participants with cancer (n=845) and without cancer (n=561). To determine the distribution of fragment lengths, cfDNA was isolated from plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen). To assess the effect of DNA concentration on fragment length distribution, samples were spiked with known amounts of DNA.

Variant Classification

cfDNA was sequenced using a NextSeq 500 instrument (Illumina), and variants were called using the GATK4 software package. Fragments were then classified into tumor-related, matched white blood cell (WBC), and ambiguous categories. The mean fragment length was calculated for each category, and statistical analysis was performed using R.

RESULTS

1057 of 1338 (79%) of cfDNA in the cancer cohort were matched to a tumor biopsy. Classification was correct for 95% of variants in tumor-matched WBC samples, 85% in matched tumor biopsies, 75% in non-matched WBC samples, and 60% in non-matched tumor biopsies. The most frequent tumor-related variants belonged to the WBC category, whereas the least frequent were matched to tumor biopsies.

CONCLUSIONS

The fragment length distributions of cfDNA can be used to accurately classify tumor-related variants. This method provides a new tool for identifying tumor-specific mutations and could be used to develop a single blood test for multiple types of cancer across stages.