Multi-Analyte Profiling Reveals Relationships Among Circulating Biomarkers in Colorectal Cancer

Daniel Deluca, Eric Ariazi, Jonathan Berliner, Adam Drake, John Dulin, Riley Ennis, Erik Gafni, Kate Niehaus, Gabriel Otte, Jennifer Pecson, Girish Putcha, Corey Schaninger, Arunish Sharma, Mike Singer, Abraham Tsoi, Jill Waters, David Weinberg, Brandon White, Imran S. Haque

Freesom Inc, South San Francisco, CA

INTRODUCTION

Blood-based tests hold great promise as cancer diagnostics, but most current tests are restricted to the analysis of a single class of molecules (e.g., circulating tumor DNA, circulating miRNA, circulating protein).

The utility to analyze multiple analytes simultaneously from the same biological sample is emerging as a significant advantage of liquid biopsy testing. The multi-analyte approach has been shown to be able to capture more complex genomic and transcriptomic changes, improve the sensitivity and specificity of each test, and expediting independent information among different biomarkers.

OBJECTIVE

We developed the most experimental and analytical algorithms for the integrated analysis of multiple analytes from a single blood sample.

METHODS

Multi-Analyte Approach (Figure 1)

- In silico blood samples were obtained from healthy individuals and individuals with non-relevant adenomas (non-AAs), advanced adenomas (AAs), and stage I-IV colorectal cancer (CRC)

- After plasma separation, multiple analytes were extracted on the following:
  - Cell-free DNA (cfDNA) extracted usingParseqâ”¢ Express Whole Blood Kit (Gentra Systems) and analyzed using Illuminaâ”¢ MiSeq Machine
  - cf-miRNA: Aligned to miRbase 21 using Bowtie 2. Fragments that aligned to annotated miRNA genomic regions were counted and normalized for depth of sequencing to produce a ΔXΔY dimensional vector per sample
  - Whole-genome methylation analysis (LINE-1): Genomic Methylation was assessed using whole-genome bisulfite sequencing (WGBS) to assess methylation at LINE-1 CpG sites
  - Plasma circulating levels of alpha-1-antichymotrypsin (AACT), C-reactive protein (CRP), and serum amyloid A (SAA1) were measured using ELISA (Abcam) by immunoassays. CNV, copy number variation; LINE-1, long interspersed nuclear element 1.

Whole blood was collected in K3-EDTA tubes, and double-spun to isolate plasma. Plasma was split into aliquots for cfDNA lcWGS, WGBS, cf-miRNA sequencing, and quantitative immunoassays.

Tumor fraction (TF) estimation

- Tumor fraction (TF) was estimated from Copy Number Variation (CNV) analysis of whole-genome bisulfite sequencing (WGBS) data from blood samples.

Significance was assessed by 1-way analysis of variance (ANOVA) followed by Sidak’s multiple comparison test. Only significant adjusted values are shown. CpG hypomethylation of LINE-1 was associated with a greater TF (P = 0.004).

CONCLUSIONS

- These data suggest that TF to covary with cancer stage, but does not have potential stage, even in early stage disease.
- We found that aberrant profiles among non-AAs, AAs, and CRC samples were more strongly associated with high TF than with late stage. This may explain the variability reported in cancer detection rates for other screening tests.
- This finding suggests that some patients with higher cancer burden may be too late for “high TF” detection to work.
- The presence of TF in CRC patient data is non-normal, including miRNA and protein, suggests that using composite biomarkers from a single sample may allow the development of clinical models that can cater to all TF.

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