Plasma Cell-free RNA Sequencing Enables Sensitive Detection of Lung Cancer

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Introduction: Blood-based liquid biopsy is a non-invasive cancer detection approach that isolates tumor-derived entities such as plasma cell-free DNA (cfDNA) and/or RNA (cfRNA) for personalized genomic profiling. While liquid biopsy testing is widely utilized for biomarker genotyping in diagnosed lung cancer cases, its adoption for early cancer detection has been limited due to concerns over sensitivity and specificity. Most liquid biopsy technologies have focused exclusively on profiling cfDNA, whereas cfRNA has remained relatively unexplored as an analyte. Plasma cfRNA can be interrogated to determine cancer-associated gene expression, and thus complement cfDNA-based liquid biopsy testing for potentially more sensitive and specific early cancer detection. We describe an amplicon-based next-generation sequencing test to assess the utility of cfRNA-based gene expression profiles for sensitive lung cancer detection across different cancer stages.

Methods: To determine lung cancer-associated gene expression signatures in cfRNA, an amplicon panel incorporating molecular barcoding for error-suppression was designed to target 24 messenger RNAs and long non-coding RNAs. RNA targets were selected from publicly available datasets based on specificity in lung cancer patients relative to normal controls. Plasma cfRNA from 40 subjects (16 normal and 24 lung cancers; 37.5% early-stage) was analyzed with the cfRNA expression panel and matched cfDNA was analyzed with a 32-gene mutation hotspot panel to detect DNA mutations. The abundance of cfRNA was estimated as the geometric mean read counts of a set of housekeeping genes, and a cancer-associated RNA expression score was obtained via summation of the relative normalized expression of all RNA markers. A similar DNA mutation score was obtained via summation of the normalized allele frequency of all cancer-associated mutations.

Results: Plasma cfDNA concentration increased in late-stage (38.5ng/mL; p=0.0001), but not early-stage lung cancers (median 10.0ng/mL; p>0.99), when compared to normal controls (median 12.3ng/mL). Conversely, cfRNA abundance increased in both early-stage (median 665 reads) and late-stage lung cancers (median 647 reads) compared to normal controls (median 244 reads) though it did not reach statistical significance (p=0.11). When examining cancer-associated signals, normal, early-stage and late-stage lung cancers harbored a median of 0.5,1, and 2 DNA mutations, respectively. In cfRNA, normal, early-stage, and late-stage lung cancers expressed a median of 0, 2, and 6 cancer-associated RNA markers, respectively. Comparison of the DNA mutation score across groups revealed a significant increase in late-stage (median 33.2; p=0.004), but not early-stage lung cancers (median 0.1; p>0.99), relative to normal controls (median 0.2). In cfRNA, the RNA expression score was elevated in both early-stage (median 5.8; p=0.01) and late-stage lung cancers (median 26.9; p<0.0001) relative to normal controls (median 0). By setting a cut-off value that maximizes performance, our cfRNA expression panel exhibits an overall 83.3% sensitivity (77.8% in early-stage and 86.7% in late-stage lung cancers) and 93.8% specificity.

Conclusion: We demonstrate that cfRNA profiling provides an independent, and potentially earlier, measure of cancer signals from cfDNA profiling. This implies that cfRNA can be a reliable analyte to complement cfDNA-based technologies for early detection of lung cancer. Further evaluation of this preliminary study is warranted to confirm the findings.