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Minimally invasive genomic and transcriptomic profiling of visceral cancers by next-generation sequencing of circulating exosomes

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Background: The ability to perform comprehensive profiling of cancers at high resolution is essential for precision medicine. Liquid biopsies using shed exosomes provide high-quality nucleic acids to obtain molecular characterization, which may be especially useful for visceral cancers that are not amenable to routine biopsies.

Patients and methods: We isolated shed exosomes in biofluids from three patients with pancreaticobiliary cancers (two pancreatic, one ampullary). We performed comprehensive profiling of exoDNA and exoRNA by whole genome, exome and transcriptome sequencing using the Illumina HiSeq 2500 sequencer. We assessed the feasibility of calling copy number events, detecting mutational signatures and identifying potentially actionable mutations in exoDNA sequencing data, as well as expressed point mutations and gene fusions in exoRNA sequencing data.

Results: Whole-exome sequencing resulted in 95%–99% of the target regions covered at a mean depth of 133–490×. Genome-wide copy number profiles, and high estimates of tumor fractions (ranging from 56% to 82%), suggest robust representation of the tumor DNA within the shed exosomal compartment. Multiple actionable mutations, including alterations in *NOTCH1* and *BRCA2,* were found in patient exoDNA samples. Further, RNA sequencing of shed exosomes identified the presence of expressed fusion genes, representing an avenue for elucidation of tumor neoantigens.

Conclusions: We have demonstrated high-resolution profiling of the genomic and transcriptomic landscapes of visceral cancers. A wide range of cancer-derived biomarkers could be detected within the nucleic acid cargo of shed exosomes, including copy number profiles, point mutations, insertions, deletions, gene fusions and mutational signatures. Liquid biopsies using shed exosomes has the potential to be used as a clinical tool for cancer diagnosis, therapeutic stratification and treatment monitoring, precluding the need for direct tumor sampling.

Key words: liquid biopsy, exosomes, pancreatic cancer, next-generation sequencing

introduction

For many visceral cancers, such as pancreatic ductal adenocarcinoma (PDAC), the availability of tissue-based companion diagnostics may be limited or precluded secondary to clinical factors such as tumor location, amount of tumor tissue-sampled or procedure-associated risk, hindering the progress of precision medicine [1]. Relatively noninvasive liquid biopsies offer a promising alternative for tumor characterization and disease monitoring. To this end, several investigators have identified tumor-specific genetic mutations in patient plasma-derived circulating cell-free DNA (cfDNA) including activating mutations in *KRAS*, *BRAF*, *epidermal growth factor receptor (EGFR)* and other cancer genes using highly sensitive targeted approaches such as digital PCR and targeted amplicon sequencing on cfDNA [2,3,4]. Recently, whole-genome and exome sequencing have been performed using the cfDNA of plasma samples in an effort to estimate tumor copy number profiles and identify actionable mutations in a more agnostic manner [5–7]. However, the extensively fragmented nature of cfDNA in circulation makes it difficult for this format to become generalizable in

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