



# Evaluation of clinical outcomes of pancreatic cancer patients using circulating nucleic acids

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The incidence of pancreatic ductal adenocarcinomas (PDAC) is increasing, and, owing to its malignant features and poor prognosis, it is becoming the second or third leading cause of cancer death in many countries. Somatic mutations in *KRAS*, *CDKN2A*, *TP53*, and *SMAD4* are frequently observed in PDAC (1). Bernard *et al.* examined circulating nucleic acid levels in liquid biopsies from 194 pancreatic cancer patients from April 2015 to October 2017 (2). Droplet quantitative digital polymerase chain reaction (ddPCR) was used to determine *KRAS* mutant allele fractions (MAFs) from circulating tumor cell DNA (ctDNA) and exosomal DNA (exoDNA) purified from a large series of prospectively collected plasma from patients. The authors showed that the baseline levels of CA19-9, a PDAC marker, and exoDNA and ctDNA affect the prognosis and overall survival of PDAC patients. In a longitude analysis, a MAF peak above 1% in exoDNA was significantly associated with radiological progression. This study indicated that longitude monitoring of exoDNA and ctDNA using liquid biopsies provides predictive and prognostic information for therapeutic intervention of pancreatic cancers with poor prognosis and metastasis.

In cancer diagnosis and clinical therapeutics, blood biomarkers are useful. In the case of PDAC, CA19-9 is frequently used. Recently, ctDNA in blood derived from tumors was found to be useful for the detection of various cancers including lung cancers, colorectal cancers, and PDAC (3-5). Early detection of genomic alterations by next

generation sequencing in local tumors and ctDNA may be useful for determining optimal therapeutic strategies and predicting the prognosis of each patient (3-5). Exosomes, secreted small extracellular vesicles ranging from 40–150 nm in diameter with a lipid bilayer membrane, contain miRNA and mRNA from tumor tissues and circulate in plasma. They mediate transfer of nucleic acids between cells (6,7). Double-stranded DNA is also found in exosomes and serves as a novel biomarker in cancer detection (8,9). Genomic DNA from exosomes could be transferred from donor cells to recipient cells (10). Specific proteins are similarly encapsulated in exosomes, and are involved in signal transfer between cells (11). In the aforementioned study by Bernard *et al.*, the authors compared the usefulness of tumor monitoring in localized and metastatic pancreatic tumors using paired exoDNA and ctDNA samples for diagnosis, prognosis, and therapeutic selection (2). One milliliter of plasma was used for ctDNA isolation, and 12 mL of plasma was used for exosome isolation. Exosomes were isolated by conventional sequential centrifugation. Patients with metastasis had a greater circulating MAF of *KRAS*. Although CA19-9 was able to predict disease progression without detectable exo*KRAS* mutant in some patients, a rise in *KRAS* MAF in exoDNA could be detected even before a rise of CA19-9 or CT imaging diagnosis in different patients. Therefore, these biomarkers should be used to complement each other.

ctDNA and exoDNA measurement in cancer patients

are valuable for predicting overall survival and progression-free survival beyond the detection and diagnosis of cancers. Combined measurement of ctDNA or exoDNA with CA19-9 and combination of ctDNA and exoKRAS MAF could predict survival. Therefore, multi-analyte approaches are useful not only for diagnosis but also for prognosis prediction and therapeutic selection for intractable PDAC. It is also of note that ctDNA is released from apoptotic or necrotic cells, whereas the majority of exoDNA is from viable cells.

Regarding exoDNA, purification of multiple samples by sequential centrifugation is not useful for high-throughput screening. Furthermore, although DNase-resistant intra-exosomal DNAs are located within exosomes and protected by a phospholipid bilayer membrane (9,10), several reports showed that exosomes carry DNase I-sensitive nucleic acid on the surface (12,13). Oncogenic genomic DNA either from inside or on the surface of exosomes could exhibit different levels of stability. In either case, sequential liquid biopsies to measure diagnostic and predictive nucleic acids such as ctDNA and exoDNA in PDAC patients have been found to be useful. Future studies using a tumor gene mutation panel to detect multiple mutated genes and ctDNA or exoDNA by liquid biopsies should be undertaken.

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### Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

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