Advancing cancer research with plasma-seq

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Early detection of cancer is critical for screening populations and improving the survival and quality of life of patients because it allows for earlier implementation of alternative therapies. Circulating tumor DNA (ctDNA) originates from apoptotic or necrotic tumor cells and is characteristic of the malignant process. ctDNA isolated from plasma offers the potential of a sensitive and specific biomarker for a host of applications, including diagnosis or early detection of tumors and prognostic information on overall or disease-free survival. Besides, it can be used to get predictive information on resistance and probability of lack of response to treatment. Recently, two groups published papers using ctDNA and plasma-seq technology to profile biomarkers for prostate cancer and to analyze copy number variations (CNVs) and DNA fragmentation patterns for glioma.

Profiling prostate cancer biomarkers

Researchers at the Karolinska Institute in Stockholm, Sweden, have authored an excellent paper on the profiling of metastatic castration-resistant prostate cancer (mCRPC) from cell-free DNA using the ThruPLEX Plasma-Seq Kit.

The authors performed a large-scale study of over 200 patients to demonstrate how cell-free DNA can be used to detect markers for prostate cancer, compared to germline DNA from white blood cells and other studies using DNA from a cancer tissue biopsy. Their comprehensive profiling of the androgen receptor (AR) revealed a continuous evolution of genetic alterations with intra-AR structural variations detected in a significantly lower fraction of first-line mCRPC therapy patients compared to fourth-line patients (Figure 1). They performed low-depth whole genome sequencing, as well as targeted enrichment to achieve greater depth on specific regions of interest. They used inputs ranging from 0.1–50 ng cell-free DNA (that is as low as 100 pg of cell-free DNA!).

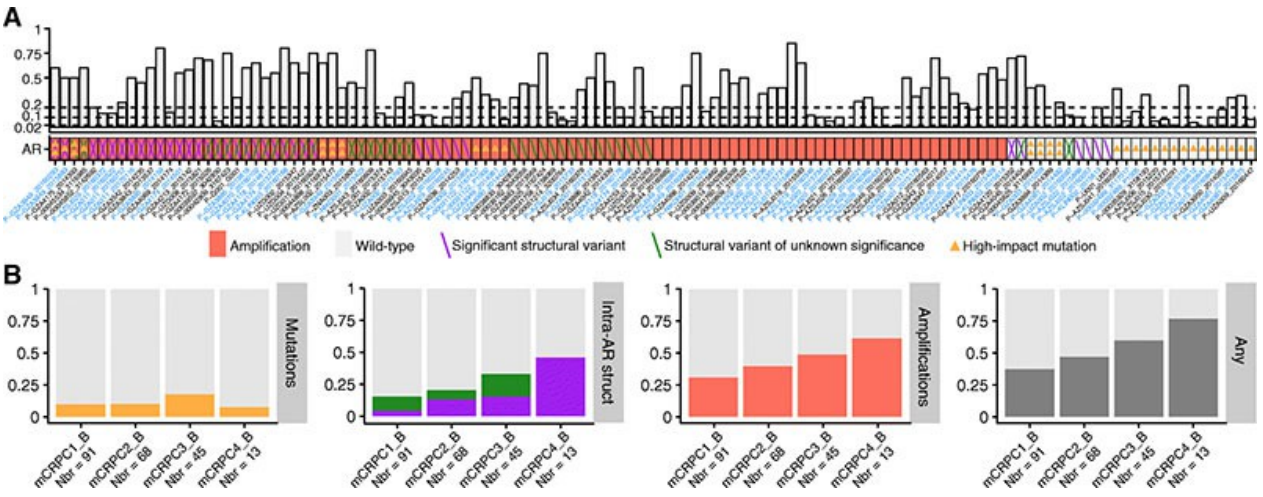

Figure 1. Androgen receptor (*AR*) alterations. Comprehensive profiling of *AR* was performed in 275 cell-free DNA samples from 177 mCRPC patients. **Panel A.** The upper part of Panel A displays the circulating tumor DNA fraction. The dashed lines at 0.02, 0.10, and 0.20 denote the cutoffs to reliably detect point mutations, loss of heterozygosity, and homozygous deletions, respectively. The lower part of Panel A displays a heatmap of the mutational landscape detected in the androgen receptor from circulating tumor DNA profiling. Type of alteration is coded according to the bottom legend. For visualization purposes, only samples with an alteration are shown here (126 samples from 89 individuals). Up to two mutations or structural variants (forward and backslashes) are displayed per sample. X-axis: cell-free DNA samples sorted according to the number of alterations detected. Patients with multiple samples are colored in blue. Asterisks indicate samples with microsatellite instability. **Panel B.** The fraction of patients with alterations in the androgen receptor are categorized by type of alteration and line of therapy. Only high-impact mutations, e.g., hotspot mutations, are shown here. Intra-*AR* structural variation is colored according to the legend in Panel A. The rightmost bar plot represents the fraction of patients with any alteration in the androgen receptor. Abbreviations: mCRPC[number], metastatic castration-resistant prostate cancer and line of therapy; _B, baseline; Nbr, number of samples profiled. Diagram and caption were adapted from Mayrhofer et al. 2018, and used under Creative Commons Attribution 4.0 International License.

Why is this important?

Cell-free DNA is a highly attractive sample type for cancer diagnosis, prognosis, and marker identification. Using cell-free DNA from plasma samples is much less invasive than obtaining a tissue biopsy and is showing great promise as a more viable technique for understanding the progression of a disease. High-throughput compatibility of the chosen sequencing chemistry for studies involving clinical samples is critical because they often require large-scale preps to minimize batch effects and improve sensitivity.

Discerning glioma CNVs and DNA fragmentation patterns

The next paper used our ThruPLEX Plasma-Seq Kit to develop a fast, low-cost assay to screen patients for glioma (tumors from glial cells found in the brain and spinal cord).

In their latest publication in *EMBO Molecular Medicine*, Cancer UK scientists demonstrated a new low-cost glioma screening method that uses cell-free tumor DNA (cftDNA) from cerebrospinal fluid (CSF) for CNV detection in patients with glioma. Their technique uses CSF cftDNA as the input (since glioma is challenging to detect in plasma) for analyzing somatic copy number alterations (SCNAs) and DNA fragmentation patterns with shallow sequencing to screen for cell-free tumor DNA (Figure 2).

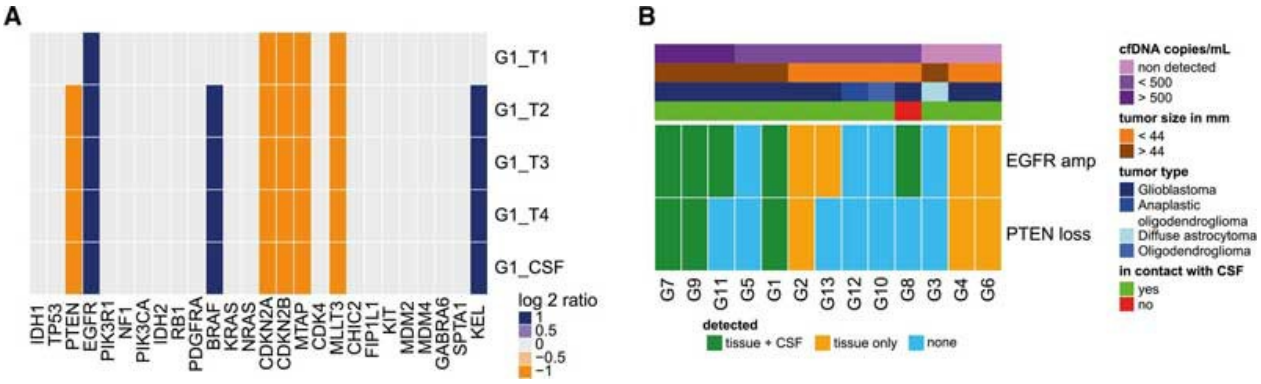

Figure 2. Detection of SCNAs in CSF is influenced by tumor grade, cfDNA concentration, and contact between the tumor and CSF. Panel A. Heat map summarizing the SCNAs detected by shallow whole genome sequencing of 28 genes of interest in tumor biopsies and CSF from patient G1 (four tumor subparts and one CSF sample). Amplifications are shown in dark blue, deletions are in orange, and copy number neutral regions are in light gray. **Panel B.** Heat map summarizing detection of *EGFR* and *PTEN* alterations in tumor tissue and in CSF samples. Shared detection in tissue and CSF is indicated in green, detection of the alteration only in tissue in orange, and non-detection in blue. The top bars indicate the cfDNA concentration (copies/ml; in a range of purples), the size of the tumors (in a range of browns), the type of glioma (in a range of blues), and whether the tumor was in direct contact with the CSF or not (based on MRI, green or red, respectively). Samples are ranked from left to right by decreasing concentration of cfDNA (copies/ml). Diagram and caption were adapted from Mouliere et al. 2018 and used under Creative Commons Attribution 4.0 International License.

Why is this important?

This assay helps to solve a significant problem: sequencing cost. By looking at copy number variation and DNA fragmentation, instead of mutations, the sequencing depth requirements are substantially lower. In other words, researchers can reliably get 'candidate' samples from low-cost screening, then follow up with deeper and more costly sequencing only on the patients with a high likelihood of disease. It is possible that this approach may be applied to other conditions or other forms of cancer. It is essential that the chosen sequencing chemistry has a fast and simple workflow that ensures quick turnaround and minimizes sequencing cost.

Future of cftDNA analysis

Recent technological advances in genetic sequencing methods have opened new avenues for cancer diagnosis and tumor monitoring that are minimally invasive. Biopsies of single sites do not address intra-tumoral heterogeneity that is captured by liquid biopsies. Using these alternative sample types could potentially eliminate the need for invasive tissue biopsies in some instances. However, analyzing liquid biopsies has its own challenges and having technologies such as ThruPLEX Plasma-seq that are optimized for cfDNA analysis with features such as a simple workflow, high-throughput compatibility, and high-quality readout with minimal sequencing depth will be instrumental in facilitating a broader application of liquid biopsies.

References

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Mouliere, F. *et al.,* Detection of cell-free DNA fragmentation and copy number alterations in cerebrospinal fluid from glioma patients. *EMBO Mol. Med.* **e9323,** (2018).

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