Circulating Tumor DNA

Subjects: Oncology
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Circulating tumor DNA (ctDNA) are small fragments of DNA, typically 150-200 bp in size, shed by tumors into blood through tumor necrosis, apoptosis and potentially through extracellular vesicles. ctDNA can also be found in other fluid spaces susch as cerebrospinal fluid and pleural fluid.

Keywords: lung cancer ; liquid biopsy ; circulating tumor DNA ; circulating tumor cells ; non-small cell lung carcinoma

1. Introduction

Circulating free DNA (cfDNA) in peripheral blood originates from normal tissue remodeling with variable contributions by tumor necrosis, apoptosis and potentially through extracellular vesicles [1][2]. cfDNA exists in a nucleosome protected 150–200 base pair sized fragments and has a half-life of approximately 2 h, allowing analysis of genomic material that reflects the current, real time status target(s) in question [3]. The concentration of cfDNA in plasma is typically low (5–10 ng/mL), and the fraction that corresponds to ctDNA can be highly varied and range from as low as 0.1% to 30% of the total cfDNA [4][5].

1.1. Methodology Considerations

With such small concentrations of DNA, special consideration is required in sample collection of liquid biopsies for cfDNA or ctDNA. Lysis of nucleated cells within samples, especially lymphoid cells, could release vast amounts of non-tumor DNA, effectively “drowning out” ctDNA and leading to false negatives. For this reason, plasma is preferred over serum, as the clotting process needed to produce serum leads to leukocyte lysis [6]. Appropriate collection and storage of samples is also crucial in order to minimize leukocyte lysis. Standard EDTA blood collection tubes are suitable for sample collection; however, samples must be processed ideally within 4 h from collection at room temperate or 24 h at 4 °C, in order to avoid significant blood cell lysis. Alternatively, proprietary collection tubes containing leukocyte stabilizing agents are available, including Streck BCT tubes (Streck Inc., Omaha, NE, USA), PAXgene tubes (Qiagen PreAnalytiX GmbH, Hilden, Germany), and cfDNA collection tubes (Roche Diagnostics GmbH, Mannheim, Germany). These collection tubes are capable of maintaining adequate sample integrity for at least 48 h, and possibly up to a week at room temperature [7][8].

1.2. Molecular Testing

The ability to detect ctDNA in a background of “normal” cfDNA poses a significant challenge. Assays need to be sensitive enough to detect the proverbial needle in a haystack, where allelic copies of mutated genes can be very low amongst the total DNA pool [4][5]. Furthermore, cfDNA and ctDNA exist in highly fragmented forms, and assay detection capabilities are required that are robust enough to detect these fragments. Many types of assays have been developed which have shown success in overcoming these challenges and are typically classified in one of two groups; targeted gene detection methods and broad panel/whole genome methods [9]. The targeted detection methods typically have higher sensitivity and are either PCR or NGS-based methods.

PCR-based methods which have shown to have the sensitivity required for analysis of ctDNA include ddPCR and BEAMing [10]. The basis of ddPCR is emulsification of the DNA within a sample into droplets containing approximately one DNA fragment each. Two chromophores are then used to distinguish between target mutation and wild-type DNA, which are detected as the samples are cycled. BEAMing is similar to ddPCR; however, biotinylation is used to bind amplified DNA to magnetic beads, allowing for direct extraction of target DNA [11]. While sensitive and cost effective, PCR-based methods do have significant limitations. Firstly, in the setting of NSCLC, they are not recommended for interrogating ALK and ROS rearrangements [10]. Secondly, PCR-based methods are able to interrogate discreet and known genetic alterations, and are limited in terms of the number of genetic targets each assay can detect. While multiplexing expands the number of targets tested per sample, the ever-increasing number of targetable genetic alterations means that broader methods will be required.

Targeted NGS platforms have been developed to allow for an expanded repertoire of targets tested. Amplicon-based and hybrid-capture-based platforms are available which provide sequencing of specific genetic targets in order to identify any actionable alterations. Amplicon-based NGS consists of using primers to amply specific portions of ctDNA, which are then sequenced. The main advantages of this method is that it requires considerably less starting material [12], and is less expensive than the hybrid-capture method [13]. Hybrid capture NGS involves using DNA or RNA fragments targeting areas of interest to purify ctDNA fragments from the remaining cfDNA. Capture-based methods provide a wider coverage with more consistent data, at the cost of requiring larger initial DNA sample, more laborious workflow, longer turnaround times and greater cost [12]. While advances in amplicon-based NGS have increased reliability and sensitivity, it is limited to known hotspots and panels are less expensive than capture NGS [14][15]. Furthermore, while it is possible to detect gene rearrangements with amplicon-based NGS, this requires the use of circulating tumor RNA (ctRNA), or incorporates multiplex PCR [16]. Hybrid capture NGS is technically more challenging; however, a well-developed and validated system could provide wider coverage, detect a wider array of genetic alterations, and provide more reliable data than amplicon-based NGS [12]. While both methods have distinct merits and limitations, other factors that have to be taken into consideration when adopting these NGS technologies into practice such as specific scope of use, resources and downstream bioinformatic capabilities.

2. Clinical Use

The use of ctDNA to guide clinical management of NSCLC presents many advantages. One of the most significant being that it is a less invasive method with a lower risk of complication for disease monitoring than serial tissue biopsy [17]. While tissue biopsies (and cytological material to an extent) remain the ‘gold standard’ for diagnostics, it remains limited with regard to serial monitoring for the development of resistance mutations or minimal residual disease (MRD) due to risks associated with tissue biopsies. ctDNA is particularly beneficial in cases where tumor biopsy is not feasible, or where obtaining a tissue biopsy would delay the initiation of treatment [18]. Furthermore, liquid biopsy-derived ctDNA can be more representative of a heterogeneous tumor or metastatic deposit, and detect actionable targets that may otherwise be missed on a single site tissue or FNA biopsy [19][20]. The use of ctDNA from peripheral blood is already widely in use in the detection of EGFR mutations in NSCLC [18]. Liquid biopsy ctDNA has also been used to detect EGFR resistance mutations such as T790M [21][22], as well as resistant mutations in ROS1 and ALK [23][24][25][26]. Furthermore, ctDNA is being assessed for its feasibility in lung cancer screening. A summary of studies assessing the utility of ctDNA in lung cancer is provided in **Table 1**.

Table 1. Summary of published studies assessing the role of ctDNA in lung cancer.

Author	Number of Patients	Platform	Main Findings
Chaudhuri et al. [27]	94	CAPP-seq (NGS)	Detectable ctDNA post-treatment preceded radiological evidence of progression in 72% of cases. Of the patients that relapsed, 94% had detectable ctDNA after treatment with curative intent.
Cho et al. [28]	36	PANAmutyper (PCR)	Factors associated with higher ctDNA in plasma included higher pathological tumor stage, nodal metastasis, solid adenocarcinoma subtype, tumor necrosis, greater tumor volume and frequent mitoses.
Li et al. [29]	26	WGS	Driver genes detected in all CSF ctDNA samples. 92.3% of patients had higher allele fractions in CSF than CSF precipitates or plasma. EGFR T790M was detected in CSF of 30.4% samples from patients who progressed on TKI.
Oxnard et al. [30]	216	BEAMing	Plasma detection of T790M was 70% sensitive. OOR and PFS were similar T790M positive tumors detected through plasma ctDNA or biopsy.
Papadopoulou et al. [31]	171	NGS	49% of NSCLC patients had at least 1 mutation detected at diagnosis by NGS. 86.1% concordance in clinically relevant mutations between ctDNA and tissue biopsy.
Sabari et al. [32]	210	ResBio ctDx-Lung	ctDNA detection lower in patients on systemic treatment. High concordance of ctDNA detected oncogenic drivers with tissue detection (91.6%).
Tailor et al. [33]	33	SureSelect All Exon V5 + UTR	Patients with malignant nodules showed a significantly higher number of somatic mutations. 82% of malignant lesions identified through mutational analysis.

Author	Number of Patients	Platform	Main Findings
Tsui et al. [34]	50	Tam-Seq PCR, digital PCR	Low levels of EGFR mutations in TKI naïve patients resulted in better PFS and OS. Pre-treatment mutations in both EGFR and TP53 correlated with worse prognosis. Progression without T790M mutation resulted in worse survival.
Uchida et al. [35]	288	NGS	EGFR exon 19 deletion sensitivity was 50.9% and specificity was 98.0%. L858R mutation sensitivity was 51.9% and specificity was 94.1%.
Weber et al. [36]	199	Cobas EGFR test	91% concordance of EGFR mutations between tissue and plasma ctDNA samples. Six EGFR mutations detected in ctDNA samples only.
Yang et al. [37]	103	Gardant360	Poor survival if >3 mutations detected in ctDNA
Zhang et al. [38]	27	NGS	Overall ctDNA and tissue concordance for driver gene mutations was 85.2%, sensitivity and specificity was 87.0% and 75%, respectively. Concordance reached 100% in cases of boney metastasis and/or concurrent TP53 mutations.
Zhao et al. [39]	111	Mutant-enriched PCR	EGFR mutation concordance between paired plasma and tissue samples was 71.2%. Sensitivity was higher for poorly differentiated tumors (77.8%) compared to well differentiated (20%) and moderately differentiated (19%) tumors.

Abbreviations: CSF: cerebrospinal fluid; EGFR: epidermal growth factor; OOR: objective response rate; PFS; progression free survival; NGS; next generation sequencing; TKI: tyrosine kinase inhibitors; OS; overall survival.

3. Conclusions

Liquid biopsies, in particular, ctDNA, have already made it to clinical use, albeit with a limited role. The technical capability to assess the relatively small amounts of ctDNA or CTCs in blood and other fluids has been quickly improving and becoming more reliable. One drawback to this rapid advancement is that there is a lack of standardization, and comparison between studies is often difficult. In regard to ctDNA, the number of targets and types of genetic alterations that we can reliably assess is ever increasing due to NGS. However, detecting low ctDNA levels such as in early LCa, post treatment or detecting MRD is still a major challenge. With increasing sensitivity and an expanding repertoire of assessable targets, clonal hematopoiesis has emerged as a factor which needs to be accounted for in the application of ctDNA. The precise pathophysiological role and implications of CTCs will require further clarification. However, in the right clinical context, CTCs appear to provide helpful information regarding risk stratification for patients with LCa. The liquid biopsy, in its many different forms, has the potential to be greatly beneficial to patients, not only through better diagnosis, prognostication and monitoring, but also by decreasing the amount of invasive procedures required.

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