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Capturing tumor heterogeneity and clonal evolution in solid cancers using circulating tumor DNA analysis

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ABSTRACT

Circulating tumor DNA analysis has emerged as a potential noninvasive alternative to tissue biopsies for tumor genotyping in patients with metastatic cancer. This is particularly attractive in cases where tissue biopsies are contraindicated or repeat genotyping after progression on treatment is required. However, tissue and plasma analysis results are not always concordant and clinical interpretation of discordant results is not completely understood. Discordant results could arise due to analytical limits of assays used for tumor and plasma DNA analysis or due to low overall contribution of tumor-specific DNA in plasma. Once these factors are ruled out, tissue-plasma concordance and quantitative levels of somatic mutations in plasma can capture tumor heterogeneity. During longitudinal follow-up of patients, this feature can be leveraged to track subclonal evolution and to guide combination or sequential adaptive treatment. Here, we summarize recent results evaluating the opportunities and limitations of circulating tumor DNA analysis in the context of tumor heterogeneity and subclonal evolution in patients with advanced cancers.

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1. Introduction

Cancer is a disease driven by acquired somatic alternations in the genome. Recent advances and lower costs of next generation sequencing have enabled comprehensive analysis of cancer genomes in research and in clinical practice. Reinforced with statistical and computational approaches and supported by functional studies, cancer genomics studies have identified >140 driver genetic alterations, vastly improving our understanding of carcinogenesis and leading to development of drugs that target specific molecular pathways (Vogelstein et al., 2013). In the clinic, tumor genotyping is increasingly used to identify patients most likely to benefit from targeted treatments. This usually involves analysis of DNA obtained from tumor biopsies for one or several recurrent cancer genes where therapeutic implications of expected mutations are well understood. In patients who have exhausted standard of care treatments and no established druggable molecular targets are identified, exome or limited gene sequencing and subsequent clinical annotation to identify drugs with potential efficacy is a subject of multiple recent and ongoing studies (LoRusso et al., 2015; McNeil, 2015). However, advanced metastatic cancers demonstrate significant intra-tumor heterogeneity such that cancer cells within a tumor or metastatic

Abbreviations: cfDNA, cell-free DNA; ctDNA, circulating tumor DNA.

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tumors within a patient successively accumulate a diverse set of mutations (McGranahan & Swanton, 2015). This repertoire of molecular alterations within each patient can outsmart treatment through selection of treatment resistant subclones and further evolution. If targeted mutations were subclonal to begin with, treatment response may be limited to the susceptible subclones resulting in shorter progression-free survival. When targeted mutations are truncal (present in the earliest cancer cells and hence shared by all clones), tumors often evolve to select for pre-existing subclones that can survive the new drug or transiently resistant cells can accumulate additional mutations that up regulate alternative molecular pathways for survival (Hata et al., 2016).

Molecular heterogeneity and treatment-driven clonal evolution limit the efficacy and duration of response to systemic treatment, leading to disease progression and treatment failure (Tannock & Hickman, 2016). In addition, they complicate subsequent choice of treatment because tumor biopsies obtained at diagnosis don't fully represent the tumor genotype at the time of disease progression. A re-biopsy is often advocated but for many patients, this is unrealistic due to concerns such as health of the patient to tolerate surgery, cost and invasiveness of the procedure and available resources in health care systems. In contrast, circulating tumor DNA analysis has been recently proposed as a potential alternative for noninvasive blood-based cancer genotyping, targeting clinical scenarios where tissue biopsies are difficult to obtain, fail to be successful or repeat genotyping is needed.

Circulating cell-free DNA (cfDNA) is comprised of short extracellular DNA fragments (approximately 160–180 bp) found in body fluids such as plasma or urine. Since most fragments are approximately the size of DNA packaged in a single nucleosome and a characteristic 10-bp step is observed in the size distribution, cfDNA is predominantly believed to result from enzymatic degradation during or after cell death (Jiang et al., 2015). Half-life of cfDNA is estimated to be ~2 h (Diehl et al., 2008). In patients with cancer, a variable fraction of cfDNA in plasma is contributed by cancer cells. These DNA fragments, known as circulating tumor DNA (ctDNA), carry tumor-specific somatic genetic alterations.

Detection, identification and quantification of ctDNA have multiple potential applications in cancer diagnostics including noninvasive genotyping (Forshew et al., 2012), monitoring of treatment response (Dawson et al., 2013) and assessment of treatment resistance and evolution (Diaz et al., 2012; Misale et al., 2012; Murtaza et al., 2013). Of these applications, plasma-based tumor genotyping has rapidly become available as a clinical test. However, there is still limited data on extensive comparison of paired tumor and plasma samples that can help establish clinical validity for ctDNA-based genotyping. Factors affecting concordance between tissue biopsies and ctDNA for a given somatic mutation include limited assay sensitivity, limited sample volumes or low overall contribution of tumor-specific DNA in plasma (such as an earlier stage patient or a mid-treatment sample when systemic burden of the tumor is low). Once technical factors are ruled out, discordance could result from intra-tumor heterogeneity because 1) a tissue biopsy did not capture a tumor clone carrying the given mutation, 2) the mutation is subclonal and not readily detectable in the ctDNA sample or 3) the tumor and plasma samples were obtained at different time points and the systemic tumor has evolved in the interim. In the current review, we explore the opportunities presented by ctDNA analysis to address tumor heterogeneity and clonal evolution and discuss technical, biological and clinical considerations in light of recent literature.

2. Technical considerations for noninvasive genotyping

Circulating DNA analysis can be affected by many technical factors that must be considered when evaluating plasma genotyping results including limited amounts of fragmented cfDNA, variable tumor fractions in cfDNA across patients, sampling inefficiencies in current analytical methods, pre-analytical variables such as time between blood collection and sample processing and background noise affecting reliability of low-abundance mutations.

Total cfDNA levels vary between individuals and are affected by a variety of physiological and pathological conditions. In healthy volunteers, a concentration of 1500–2500 haploid genome copies/mL of plasma (5–8 ng/mL) has been reported. From a routine 10 mL blood sample that may yield 4–5 mL of plasma, this limits the total available analyte to ~ 10,000 haploid genome copies (Devonshire et al., 2014). However, total cfDNA levels can be higher during diverse conditions such as after strenuous exercise, during pregnancy, in patients with rheumatologic disease, after a myocardial infarction and in patients with cancer.

Fractional contribution of tumor-specific ctDNA in plasma also varies between cancer patients and is affected by cancer type, disease stage and time of sampling (relative to treatment response). In a survey of 640 patients across multiple cancer types, Bettegowda et al. observed ctDNA was detectable in 82% of patients with metastatic stage IV cancer compared to 47% with stage I cancers (Bettegowda et al., 2014). In the same study, patients with breast and colorectal cancer were found to have much higher ctDNA concentration compared to patients with pancreatic cancer and glioblastoma. Even within patients with metastatic cancers where genotyping for actionable mutations is most relevant, ctDNA fractions in plasma can vary widely. For example, in one study of patients with metastatic colorectal cancer, pre-treatment tumor DNA fraction in plasma ranged from 0.5% to 64.1% with a median of 10.5% (Thierry et al., 2014).

Sampling and process inefficiencies of current methods further limit the effective amount of cfDNA analyzed. Ligation-based DNA sequencing typically achieves a conversion rate of <50% when input DNA amounts are limited to <50 ng (Newman et al., 2016). On the other hand, PCR based approaches, such as digital PCR or amplicon sequencing, are limited by the interaction between PCR amplicon size and average size of input DNA fragments. Effective sampling of cfDNA using a PCR amplicon of known size can be quantitatively predicted. For example, assuming cfDNA is randomly fragmented to an average size of 166 bp, a 120 bp amplicon would at best capture 27% of all input material. In contrast, a 70 bp amplicon would at best capture 58% (Lanman et al., 2015).

Delays between blood collection and processing to isolate and store plasma can cause lysis of peripheral blood cells, erroneously increasing total cfDNA levels measured in plasma. This lowers effective mutant DNA fraction in the sample, making it more difficult to detect ctDNA specially if the analytical method has limited sensitivity for variants with low allele fractions. Similarly, the use of serum instead of plasma can lead to wide variability in mutant signal introduced at the pre-analytical stage (Bronkhorst, Aucamp, & Pretorius, 2015). Optimized protocols and purpose-made blood collection tubes for cfDNA analysis can help overcome these variations (Norton, Lechner, Williams, & Fernando, 2013). Plasma mutation levels are usually reported either as absolute copies of mutant DNA fragments per unit volume of plasma or as mutant allele fraction (mutant copies as a fraction of total cfDNA). While the latter allows normalization for total cfDNA levels that could physiologically vary between patients, it must take into account any artifacts introduced by peripheral cell lysis during sample processing. PCR methods for total cfDNA measurement can also be affected if the targeted genomic loci undergo copy number alterations in the tumor.

With median ctDNA fractions of 0.5% in patients with advanced cancers, plasma genotyping assays must be optimized to distinguish true signal from background noise and demonstrate diagnostic accuracy at any proclaimed limit of detection (Lanman et al., 2015). Sequencing and PCR noise can be introduced at multiple stages of analysis including polymerase errors during amplification, DNA damage during library preparation, fixatives or preservatives used during blood collection, assay or probe non-specificity or sequencing quality. This is particularly relevant for plasma genotyping applications where additional confirmation from corresponding tumor samples is unavailable. Thresholds