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Review

Utilizing circulating tumour DNA in radiation oncology

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ABSTRACT

Emerging technologies for detection of circulating tumour DNA (ctDNA) are expanding the possibilities for clinical impact to patients with localized, potentially curable cancer. For such patients, ctDNA analysis could aid in prognostication, prediction of treatment response, longitudinal monitoring for adaptive treatment, and evaluation of minimal residual disease. Radiation oncologists currently have few tools at their disposal for predicting or rapidly assessing treatment efficacy. By reflecting the genetic and epigenetic makeup of tumours as well as dynamic changes with treatment, ctDNA as a biomarker for radiation response could enable new personalized treatment approaches. In this review, we will discuss recent advances in ctDNA technologies and potential clinical applications of ctDNA analysis throughout the therapeutic course. Furthermore, we will consider how ctDNA analysis could someday guide radiotherapy prescriptions by revealing differences in tumour radiophenotype.

Biomarkers in radiation oncology

Recent technological advances have led to dramatic improvements in precision treatment of cancer patients with ionizing radiation. Intensity modulation, on-board imaging, and motion management techniques have enabled improved dose distribution from external beam radiotherapy (RT), minimizing dose delivered to surrounding normal tissue and better targeting of the tumour [1]. Unlike these improvements in the *physical* delivery of RT, our understanding of the *biological* basis of RT efficacy has been under-utilized for the purpose of augmenting the therapeutic index.

Headway is now being made in the discovery of biomarkers for personalized RT. Both *prognostic* and *predictive* biomarkers could help guide treatment and stratify patients who might benefit from treatment intensification or de-escalation [2]. Such biomarkers may include intrinsic radiosensitivity, HPV status, hypoxia, cancer stem cell surrogates, and repopulation [3]. These parameters reflect the biological characteristics that have been shown to confer differences in tumour response to radiation. However, there remains a need for practical approaches to directly assess these parameters in clinical settings. This may take the form of tissue-based analytes, functional imaging parameters, or blood-borne molecules. As imaging- and blood-based biomarkers are amenable to serial noninvasive assessment before, during, and after the RT course, these have generated much excitement and attention from the research community.

Characteristics of circulating cell-free DNA

The presence of fragmented cell-free DNA (cfDNA) within peripheral blood has been recognized for decades [4]. The majority of cfDNA is derived from non-malignant cells, typically haematopoietic in origin [5–8]. Tumour cells can also release DNA into the circulation that is termed circulating tumour DNA (ctDNA). The levels of both total peripheral blood cfDNA and tumour-specific ctDNA in the plasma of individuals can vary considerably [9], even between patients with the same tumour type [9,10]. For example, ctDNA as a fraction of total cfDNA was found to range from 0.01 to 1.7% in colorectal cancer, 0.02–3.2% in NSCLC, and up to 47% in multiple myeloma [11–13]. The concentration of ctDNA is thought to correlate to some extent with tumour size and the rate of tumour cell death and is also influenced by factors that affect access to the systemic circulation (e.g., the blood brain barrier).

The main mechanisms of cfDNA release into the circulation are through apoptosis and necrosis [6,14–16]. Active secretion of cfDNA has also been proposed as an alternative mechanism but its physiological relevance remains uncertain. Once in the circulation, cfDNA that is shed by tumours may be taken up by resident and circulating phagocytes, degraded by circulating nucleases, and excreted in urine, stool, saliva, and other bodily fluids.

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Increased tumour burden and cellular shedding results in inefficient clearance of ctDNA, eventually leading to its accumulation in the circulation [6,11,14].cfDNA displays a characteristic fragment size distribution centred at approximately 166 base pairs. Fragments of this length correspond to the DNA wrapped around a nucleosome plus its linker. This feature reflects the protective effect of histones against the activity of endonucleases cleaving DNA at exposed sites within the chromatin of cells undergoing apoptosis [6,14,17]. Thus, apoptotic cells have been proposed to be the predominant source of cfDNA [5,11,14,17–21]. Although DNA fragments generated through necrosis are initially much larger in length (>10,000 base pairs) [14,22], circulating nucleases cleave the large DNA fragments into fragments that are of similar size or even smaller than those derived from apoptotic cells. For example, Jiang et al. found that in a cohort of 90 hepatocellular carcinoma patients, higher tumour burden and elevated levels of tumour-derived ctDNA were both correlated with DNA fragments shorter than 166 base pairs [23].

Once released into the circulation, cfDNA is short lived. The half-life of elimination for cfDNA has been reported to range from 16 min to 2 h [18,24–26]. This property allows ctDNA to depict a 'real-time' snapshot of tumour changes and disease burden. Thus, ctDNA may enable more rapid evaluation of tumour dynamics compared to conventional protein or imaging biomarkers [15,18,27].

Methods for measuring ctDNA

The use of ctDNA as a liquid biopsy for cancer has accelerated over the past two decades due to advances in methodologies for detecting DNA somatic variants at very low allelic fractions (<0.1–1%) [15,28]. Prior to this, the implementation of ctDNA as a clinical biomarker was critically limited due to substantial challenges in detecting the 'signal' (i.e., cancer-specific DNA variants) from the 'noise' (i.e., the vast background of cfDNA released from non-malignant cells). The advent of highly sensitive genomic tech nologies—specifically, PCR-based quantitative approaches and next-generation sequencing (NGS)-based detection methods—has opened the door to a broader array of clinical applications for ctDNA analysis [11,15,18].

Initial approaches to ctDNA analysis relied on allele-specific PCR methods for detection of hotspot mutations in plasma or serum, with limited sensitivity and inconsistent detection [29–31]. Digital PCR technologies, including droplet digital PCR and BEAMing, offer improved sensitivity and precision for detection of mutations with low allele fraction [11,32–36]. These methods enable investigation of a small number of mutations in parallel but are not capable of simultaneous interrogation of large segments of genes or intergenic regions. In contrast, NGS techniques allow detection of multiple genomic regions of interest through targeted sequencing using PCR amplicons or hybrid capture methods [12,35,37]. By detecting multiple cancer-specific somatic variants simultaneously, the ctDNA signals can be more accurately distinguished from non-cancer noise [12]. Methods aimed to suppress errors introduced by PCR and/or sequencing through molecular barcoding and other approaches can further improve the detection threshold of mutations with allelic fractions below 0.1% [38,39]. These techniques have enabled highly sensitive detection of ctDNA in patients and are now being advanced within clinical settings.

Detecting and quantifying ctDNA – Clinical applications in RT patients

Assessment of RT efficacy typically relies on medical imaging modalities including computed tomography, positron emission tomography, and magnetic resonance imaging [40–42]. Despite the ubiquity of these approaches, sensitivity and specificity is often insufficient for accurate determination of treatment response. For example, lung radiotherapy often causes inflammatory pulmonary infiltrates and fibrosis that is difficult to distinguish from residual/recurrent cancer. In certain contexts, blood-based biomarkers are able to offer greater sensitivity than medical imaging to assess treatment response. A prime example is prostate-specific antigen (PSA) in prostate cancer, which has become the standard response biomarker in this disease [43]. However, even PSA is imperfect owing to its lack of cancer specificity and to its uncoupling from cancer burden during androgen deprivation therapy, which is commonly administered during and following radiotherapy.

Other than prostate cancer, most cancer types do not have any reliable blood-based response biomarker. Because ctDNA is by nature cancer-specific, it has the potential to provide similar or even greater utility for multiple cancer types as PSA has for prostate cancer. In fact, ctDNA detection has already shown initial promise in comparisons with conventional protein biomarkers and imaging modalities for monitoring disease progression and treatment response [12,18,27,44,45]. In this section, we will review potential clinical applications of ctDNA detection and quantification throughout the therapeutic course (Fig. 1).

Pre-treatment ctDNA detection for prognostication and risk stratification

Seminal studies demonstrating the potential clinical applications of ctDNA were conducted in patients with nasopharyngeal carcinoma (NPC). NPC is strongly associated with Epstein-Barr virus (EBV) infection, endemic to Southeast Asia where almost all cases harbour the virus [46]. An early study investigated the prognostic utility of pre-treatment plasma/serum EBV DNA load by quantitative PCR in patients with NPC [47]. Patients with higher median plasma EBV DNA concentration within the first year after treatment had increased risk of recurrence and metastasis, independent of clinical disease stage [47]. In a subsequent study, Lin et al. [48], showed that advanced NPC patients with pre-treatment levels of EBV DNA greater than 1500 copies per millilitre had unfavorable survival and relapse rates. These studies, and many others since, have helped make plasma EBV DNA one of the most well established blood-based DNA tumour biomarkers, and studies on NPC continue to guide the entire liquid biopsy field.

More recently, ctDNA has been investigated in the context of other virally related cancer types. The establishment of a causal relationship between human papillomavirus (HPV) infection and a subset of oropharyngeal carcinomas (OPC) marked the potential for HPV DNA to act as blood-based biomarker to assess response and detect recurrence in these patients [49]. Capone et al. compared the use of conventional PCR to quantitative PCR (qPCR) to detect HPV DNA in the sera of OPC patients [50]. Quantitative PCR detection using primers targeting the E7 open reading frame resulted in measurable levels of HPV DNA in two additional sera samples (6/65 samples) not previously detected by conventional PCR (4/65 samples) [50]. More recent studies have shown varying levels of detection sensitivity. Cao et al. detected HPV DNA in 65% of pre-treatment plasma samples from HPV-positive OPC patients [51]. Ahn et al. showed that combining detection of HPV DNA in saliva and plasma increased sensitivity of pre-treatment HPV detection to 76% from 52.8% and 67.3% respectively [52]. However, Dahlstrom et al. found that pre-treatment serum HPV did not have clinical significance as a biomarker for disease recurrence [53]. The inconsistencies between these studies suggest that HPV ctDNA detection in OPC may be less sensitive than EBV ctDNA in NPC.