Circulating DNA: an information treasure trove for cancer medicine

Fragments of DNA that are derived from dead tumour cells and shed into a patient's blood have been utilised as biomarkers for the diagnosis and prognosis of liver cancer.

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Successful cancer treatment is dependent on many factors, including the correct identification of the type of cancer (diagnosis) and an accurate prediction of its likely course (prognosis). A number of biomarkers are widely used for the diagnosis of cancers today. However, identifying and employing the most accurate biomarker has proven difficult, particularly for cancer detection. In this issue of *Nature Materials* [1] the group led by Kang Zhang compared the epigenetic profiles of normal *versus* cancer circulating tumour DNA to select and validate biomarkers for the diagnosis and prognosis of hepatocellular carcinoma (HCC).

Both healthy and cancer cells release genomic DNA (gDNA) into the blood stream during cell death through apoptosis or necrosis, or by active secretion in the form of short cell-free DNA (cfDNA) fragments [2]. In cancer patients, only a small proportion of the total cfDNA is derived from the tumour and referred to as circulating tumour DNA (ctDNA). From a clinical perspective, this allows tumour DNA from anywhere in a patient's body to be detected and monitored as ctDNA in the plasma obtained from peripheral blood though a minimally invasive blood draw or liquid biopsy [3]. Despite their sparseness, ctDNAs have been detected for a variety of cancers even at an early stage of the disease progression [4]. However, such a diagnostic procedure is not without challenges due to minute amounts of ctDNA, particularly if the analysis involves epigenetic changes [5]. Epigenetic changes refer to chemical modifications (such as DNA methylation) of the genome and its functional configuration (chromatin). Collectively, epigenetic modifications confer the required plasticity for cellular differentiation which, when deregulated in cancer, becomes one of its major hallmarks [6]. Although ctDNA carries the relevant genetic as well as epigenetic information, simultaneous detection of genome-wide DNA methylation marks in cfDNA has been limited by the requirement of large amounts of starting material for target capture.

In their latest research, Xu *et al*. demonstrated the power of targeted bisulfite sequencing for the detection of ctDNA methylation markers for the diagnosis and prognosis of HCC in a cohort of 1098 HCC patients and 885 healthy control subjects. They initially screened approximately 450,000 cytosine guanine dinucleotide sites (CpGs) across the genome for differentially methylated positions (DMPs) between HCC tumour DNAs (tDNAs) from The Cancer Genome Atlas and gDNAs derived from blood samples of heathy controls (Figure 1). They then designed capture probes for the top 1,000 DMPs, of which less than half were successfully amplified and further tested in matched sets of tDNA, gDNA and ctDNA from patients and healthy controls. Identification of blocks of co-methylated CpGs allowed the authors to further refine the panel. Finally, they used machine learning algorithms to extract a panel of 10 DMPs that were capable of discriminating between HCC and healthy controls as well as potentially confounding indications such as viral infection, cirrhosis and fatty liver. The resulting combined diagnostic score (cd-score) compared favourably to that achieved by current clinical practice using alpha-fetoprotein (AFP) as a biomarker and reached a validated sensitivity of 90.5% and specificity of 83.2%. This indicates that the cd-score based test is suitable for the diagnosis of HCC using liquid biopsies and was successfully assessed for predicting tumour load, treatment response and HCC staging. In a parallel approach, the authors also established a combined prognostic score (cp-score) based on a different set of 8 DMPs and a comprehensive survival analysis. The resulting validated cp-score based test was able to predict high *versus* low prognostic risk independently of other factors and, again, was superior to the current clinical practice using AFP.

Diagnosis of cancer is challenging due to the heterogeneity of the disease in terms of identification of accurate biomarkers for a specific cancer type. As we move into an era of personalised and precision medicine which involves deep and multi-omic profiling of each cancer patient, this current limitation can be addressed through large data sets and more advanced machine and deep learning algorithms, presuming there is a will among all concerned to share the required information more openly than is currently practised [7]. As the current study has successfully demonstrated, the combination of a range of platforms is inevitably essential for more precise and informed diagnosis of cancers. A recent study utilised a combination of conventional tumour DNA assays, bioinformatics tools, as well as cognitive computational systems, for the analysis of deep cancer genomic datasets to identify actionable variants and therapeutic options in a clinically timely manner for individual patients [8].

Despite of the limitations of these studies, such as the need for longer-term patient surveillance, they take a major step forward in highlighting the progress of cancer diagnosis, predicting prognosis and identifying patients that would likely require aggressive therapeutic options. With our aging populations comes the dramatic increase in the incidence of cancer. According to Cancer Research UK, one in two individuals will develop cancer at some point in their lives [9]. However, the use of biomarkers such as ctDNA methylation markers and prediction of prognosis, the goal of defeating cancer, or at least turning it into a treatable disease through early detection is evidently coming within reach.

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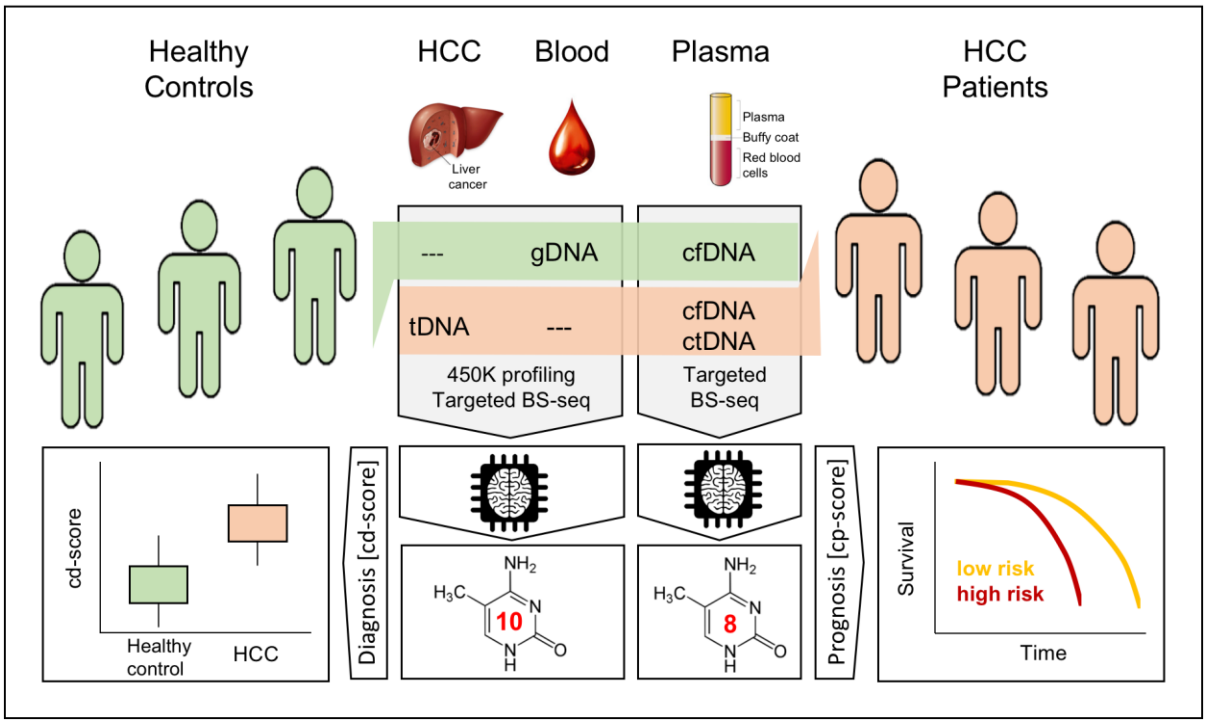

Figure 1: Study design and innovations. The upper panel shows different types of DNA (genomic DNA (gDNA) tumour DNA (tDNA), cell-free DNA (cfDNA) and circulating tumour DNA (ctDNA)) that were analysed using 450K profiling and/or target bisulfite sequencing (BS-seq). The lower panel shows the innovative use of machine learning algorithms (depicted by the brain-in-a-chip icon) for the identification of 10 and 8 DNA methylation biomarkers (depicted as 5-methylcytosines) for HCC diagnosis and prognosis, respectively.