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Review

Applications of DNA fragments in Blood Samples

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ABSTRACT

In the blood of a cancer patient, in addition to the usual cell types, other components like Circulating Tumour Cells (CTCs) and circulating cell-free DNA (ccfDNA, especially circulating tumor DNA, ctDNA), circulating cell-free RNA (ccfRNA), can also be found. On biochemical analysis of this blood (Liquid biopsy, LB) can give the clue for the presence of a type of cancer the patient is suffering. The LB is a fast, non-invasive, simple and accurate technique for a clinical diagnostic test. On the other hand, conventional methods of cancer diagnosis, such as histopathological examination, biochemical analysis of blood and DNA sequencing are cumbersome invasive and time-consuming. Advantages of liquid biopsy over conventional biopsies were discussed.

Keywords: *Clinical Diagnosis; (CTC) Circulating Tumor Cells; Oligonucleotides; Liquid Biopsy.*

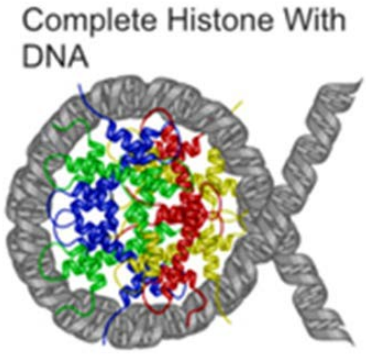
INTRODUCTION

Blood is a special type of tissue made up of plasma and specialized cells. Plasma makes the blood fluid, which facilitates it to flow in arteries and veins easily and carry the specialized cell to the site where these have their specific functions. Thomas Ashworth[1] as early as 1869 observed in the blood of cancer patients, in addition to the normal component, some other cells, which were identified as cancer cells. Normally, DNA is enclosed in membranous organelles, the nucleus, where it is transcribed into RNA, which in turn translates into protein. In addition to the nucleus, mitochondria and chloroplast also have circular DNA with a limited number of genes. In 1968, we have localized DNA in the ooplasm of Bufo by using Feulgen reagent. The unfertilized degenerating eggs show a DNA fragmentation scattered all over the ooplasm.[2] Until recently, however, nobody suspected free-floating DNA in the blood plasma; Williamson in 1970[3] was the first one who indicated the presence of oligomeric fragments of DNA in primary neonatal liver cultures, With the refinement of DNA detection and sequencing techniques (see, Gupta, P D, Editorials[4,5] these fragments are also observed in the blood; this brings a new plethora of applications in clinical diagnostics. This finding was consistent with the hypothesis that these DNA fragments were a specific degradation product of nuclear DNA.[6]

During apoptosis (programmed cell death) DNA undergoes fragmentation by the action of endonucleases and comes out of the nucleus into the cytoplasm from where these fragments are released in the blood on cell disintegration. DNA fragmentation is a biochemical hallmark of apoptosis.[7,8]

In dying cells, DNA is cleaved, fragmenting the chromatin into nucleosomal units (Figure 1, Graphic structure and composition of the nucleosome (After Nagata, 2000, Exp Cell Res.256 (1):12-18)) which are multiples of about 180-bp oligomers and appear as a DNA ladder when run on an agarose gel.[6] The enzyme responsible for apoptotic DNA fragmentation is the Caspase-activated DNase (CAD). Another protein, the Inhibitor of Caspase Activated DNase (ICAD), normally inhibits CAD. During apoptosis, the apoptotic effector caspase, caspase 3, cleaves ICAD and thus causes CAD to become activated.

Figure 1. Graphic structure and composition of the nucleosome



CIRCULATING TUMOUR CELLS (CTCS)

Thomas Ashworth[1] in 1869 after observing CTCs postulated, "*Cells identical with those of kijycancer itself being seen in the blood may tend to throw some light upon tuhe mode of origin of multiple tumours existing in the same person*". CTCs are cells shed into the vasculature or lymphatics from a primary tumour and are carried around the body through the blood circulation to all parts of the body and are responsible for the subsequent growth of additional tumours (metastases) in distant organs. Due to this mechanism, the vast majority of cancer patient dies. The

detection and analysis of CTCs in the blood can aid early patient prognoses and suitable strategies can be evolved for the management of cancer treatments.[9,10] A thorough comparison of the morphology of the circulating cells to tumour cells from different lesions led Ashworth to conclude that "*One thing is certain, that if they [CTC] came from an existing cancer structure, they must have passed through the greater part of the circulatory system to have arrived at the internal saphena vein of the sound leg*".[1] The importance of CTCs in modern cancer research is realised in the mid-1990s with the demonstration that CTCs exist early on in the course of the disease in almost all the cases.[11,12] These results were made possible by exquisitely sensitive magnetic separation technology employing Ferrofluids (colloidal magnetic nanoparticles) and high gradient magnetic separators invented by Paul Liberti and motivated by theoretical calculations by Liberti and Leon Terstappen that indicated very small tumours shedding cells at less than 1.0% per day should result in detectable cells in the blood. A variety of other technologies has been applied to CTC enumeration and identification since that time.

Modern cancer research has demonstrated that CTCs derive from clones in the primary tumour, validating Ashworth's remarks.[11] However, the single-cell analysis demonstrated, both, morphological and biochemical heterogeneity at the single-cell level, therefore it was concluded that the CTCs reflected both the primary biopsy and the changes seen in the metastatic sites.[12,13]

Compared to CTCs, tissue biopsies are more cumbersome for accurate diagnosis of primary and metastatic tumour type. CTCs thus could be considered a "liquid biopsy" which reveals metastasis in action, Analysis of blood samples found a propensity for increased CTC detection as the disease progressed in individual patients.[6,12]

SOURCES OF DNA IN THE BLOOD

Now it is a well-known fact that during apoptosis CTCs release DNA fragments in the bloodstream by the well-known mechanism (see above). Many authors have described[2,3,7,8,14-17] extracellular DNA and RNA which come in blood circulation during cell disintegration.

LIQUID BIOPSY: THE CURRENT STATUS

Liquid biopsy is not really a biopsy as they are blood tests that do not require a biopsy of solid tissues. This technological development could make it possible to diagnose and manage cancer from repeated blood tests rather than from a traditional biopsy. The emergence of liquid biopsies and non-invasive progression monitoring has resulted in the development of much new Cell-Free DNA (cfDNA) assays. The credit goes to Johann S. de Bono and his team.[18] The technology involves the identification of fragments of DNA circulating in the blood Analyses of circulating DNA can be used to monitor response to treatment, assess the emergence of drug resistance, and quantify minimal residual disease. As a result, liquid biopsies are becoming increasingly popular in cancer research.[19] A liquid biopsy of circulating tumour-specific DNA fragments has been validated and approved by the FDA as a useful prognostic method for various types of cancer. However, at present, the molecular landscapes of solid tumours are established using surgical or biopsy tissue samples. Nevertheless, liquid biopsies have a great future.

ADVANTAGES OF LIQUID BIOPSY OVER THE CONVENTIONAL TISSUE BIOPSY

There are two types of essays that can be performed in the blood (liquid biopsy) in which one can either measure

(1) Circulating tumour cell or

(2) cell-free circulating tumour DNA.

Liquid biopsies provide many advantages over conventional tissue biopsy-based genomic testing. There are three key areas where liquid biopsies are making a positive impact on growth in precision medicine. It helps patients in targeted therapy, early diagnosis and monitoring response and minimal residual disease. These all have the potential to not only improve patient well-being and outcome but decrease time and cost; however, one of the greatest challenges in liquid biopsy assays, is limit of detection and faithful standards for comparison.

By detecting and quantifying genomic alterations in CTCs (Circulating Tumour Cells) and cell-free DNA in blood, liquid biopsy can provide real-time information on the stage of tumour progression, treatment effectiveness, and cancer metastasis risk. These tests analyse fragments of tumour-cell DNA (see above) that are continuously shed by tumours into the bloodstream. These methods provide a non-invasive alternative to repeat invasive biopsies to evaluate the mutations in cancer and plan individualized treatments. In addition, because cancer is a heterogeneous genetic disease[19] and excisional biopsies[20] provide only a snapshot in time of some of the rapid, dynamic genetic changes occurring in tumours, Analysis of individual CTCs demonstrated a high level of heterogeneity seen at the single-cell level for both protein expression and protein localization and the CTCs reflected both the primary biopsy and the changes seen in the metastatic sites.

Analysis of Cell-Free circulating tumour DNA (cfDNA) has an advantage over circulating tumour cells assays in that there is approximately 100 times more cell-free DNA than there is DNA in circulating tumour cells. These tests are moving into widespread use when a tissue biopsy has insufficient material for DNA testing or when it is not safe to do an invasive biopsy procedure.

Such tests may also be useful to assess whether malignant cells remain in patients whose tumours have been surgically removed. Another potential use is to track the specific DNA mutations driving a tumour.[21] Many new cancer medications block specific molecular processes. Such tests could allow easier targeting of therapy to tumour. Such tests may also be useful to assess whether malignant cells remain in patients whose tumours have been surgically removed. Yet another potential use is to track the specific DNA mutations driving a tumour. Many new cancer medications block specific molecular processes. Such tests could allow easier targeting of therapy to tumour. Analysis of cell-free circulating tumour DNA (cfDNA) has an advantage over circulating cells assays in that there is approximately 100 times more cell-free DNA than there is DNA in circulating tumour cells. These tests are moving into widespread use when a tissue biopsy has insufficient material for DNA testing or when it is not safe to do an invasive biopsy procedure.

Blood tests are easy and safe to perform and multiple samples can be taken over time. By contrast, analysis of solid tumours necessitates invasive procedures that might limit patient compliance. The ability to monitor the disease progression over time could facilitate appropriate modification to a patient's therapy, potentially improving their prognosis and quality of life.[22] The important aspect of the ability to prognose the future progression of the disease is elimination (at least temporarily) of the need for a surgery when the repeated CTC counts are low and not increasing; the obvious benefits of avoiding the surgery include avoiding the risk related to the innate tumour-genericity of cancer surgeries. To this end, technologies with the requisite sensitivity and reproducibility to detect CTCs in patients with metastatic disease have recently been developed excisional biopsies are invasive, cannot be used repeatedly, and are ineffective in understanding the dynamics of tumour progression and metastasis.[23,24] Moreover, tissue-based tumour profiles are, however, subject to sampling bias, provide only a snapshot of tumour heterogeneity, and cannot be obtained repeatedly.

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