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Liquid Biopsies for Cancer Management
CIRCULATING TUMOR DNA IN BLOOD AS A LIQUID BIOPSY FOR CANCER

The existence of cell-free DNA molecules (cfDNA) circulating in human blood was described in 1948, but it was thirty years later when a higher concentration of cfDNA in cancer patients than in individuals without the disease was reported. This circulating tumor DNA (ctDNA) belongs to the pool of the total cfDNA in blood, but it specifically derives from tumors. In individuals without cancer, fragments of DNA are released into the blood because of cell death (apoptosis), but the concentration of cfDNA is low due to clearance of dead cells by phagocytes. However, tumor patients generally have significantly higher levels of cfDNA because of the high turnover of cancer cells, through both apoptotic and necrotic processes.

Interestingly, both the concentration of ctDNA in blood and the fraction of patients with detectable levels of ctDNA correlate with tumor stage: 47% of patients with cancer at stage I had detectable ctDNA; this fraction increased to 55%, 69% and 82% for patients at stage II, III and IV, respectively (Figure 2A). However, the frequency of patients with detectable ctDNA varies across cancer types (Figure 2B).

Figure 1. Tumor cells release fragments of DNA (ctDNA) into the bloodstream. Modified from Wan et al.

Figure 2. A) Fraction of patients with detectable ctDNA increases with tumor stage. B) Fraction of patients with detectable ctDNA in advanced malignancies depends on cancer type. Modified from Bettegowda et al.
As mentioned above, solid biopsies are the standard way of profiling genetic alterations in tumors for treatment management. Moreover, they provide information about histological issues and need a short operating time. However, solid biopsies show some limitations that can be overcome with the use of the liquid biopsy. In this sense, Krishnamurthy and co-workers have stated that ctDNA already complements and could eventually supplant solid biopsies. Indeed, a strong concordance in detecting mutations between solid biopsies and ctDNA has been reported: 83.3% in metastases biopsies and 78.5% in primary tumor biopsies. Moreover, solid biopsies are not easily obtained and imply risk and pain for the patient; liquid biopsies, on the other hand, are minimally invasive allowing repeats and are less expensive. Finally, another advantage of ctDNA over tissue biopsies is that ctDNA can detect intratumoral heterogeneity, which is unappreciated by single-site solid biopsies. This heterogeneity could explain the small discordance found when comparing solid versus liquid biopsies.

Cancer antigens are proteins used to assess a therapeutic response and detect relapse in a non-invasive way. Some very well-known examples of such cancer biomarkers are PSA in prostate, CA19-9 in pancreatic, CEA in gastrointestinal or CA-125 in ovarian cancer. However, these cancer antigens are proving to be unreliable for treatment response monitoring, and they are also elevated in individuals without cancer. ctDNA has been confirmed to contain DNA mutations of both primary and metastatic lesions, such as point mutations, copy number variations and insertions/deletions. Nowadays, personalized cancer therapy – based on genetic alterations – relies on the acquisition of tumor tissue via biopsy and posterior targeted genome sequencing, either before therapy initiation or after resistance appearance. Such profiling of genetic alterations in the ctDNA is defined as “liquid biopsy”. Different studies have shown two main applications of ctDNA liquid biopsies: the assessment of specific mutations that can direct patient management (clinically actionable), and the monitoring of the response and resistance to therapy.

1) Assessment of specific mutations

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2) Monitoring of the response and resistance to therapy

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EXOSOMES AND TUMOR-EDUCATED PLATELETS AS LIQUID BIOPSIES FOR CANCER

cDNA is not the only entity in blood with information about tumor genetic alterations. In fact, the information obtained from the cDNA can be further complemented through the analysis of mRNA contained within vesicles (exosomes) or sequestered in tumor-educated platelets.

1) Exosomes

Exosomes are small sized extracellular vesicles of endocytic origin. They play a key role in cell-to-cell communication by carrying constituents of the cell of origin, including RNAs, DNAs and metabolites. However, this communication is not only involved in the regulation of normal physiological processes, but also in pathological events such as cancer. Exosomes are secreted from most cell types and released into different fluids of the body (e.g. blood), and they have a great potential to serve as a liquid biopsy tool in cancer. Indeed, exosomes derived from tumor cells carry the cargo reflective of their genetic alterations, and the analysis of the mRNA present (Figure 4) can provide information concerning gene fusions, aberrant splicing forms and mutations due to RNA editing.

![Figure 3. Tumor cells release exosomes containing tumor mRNA into the bloodstream. Modified from Wan et al.](image)

2) Tumor-educated platelets

A second source of tumor-derived RNA are tumor-educated platelets. Blood platelets are the second most-abundant cell type in peripheral blood. They are circulating cell fragments with no nucleus that originate in bone marrow from megakaryocytes, and that are involved in hemostasis and wound healing initiation. But more recently, platelets have also emerged as players in tumor growth and cancer progression. Interestingly, platelet RNA profile is affected in almost all cancer patients and is dependent on several factors, such as the transcriptional state of the bone-marrow megakaryocyte (platelets are anucleated, so their mRNAs are derived from megakaryocytes during platelet origination), possibly queue-specific pre-mRNA splice events in response to signals released by cancer cells and the tumor microenvironment, and the transfer of spliced mRNA from tumor cells (Figure 5). Indeed, confrontation of platelets with cancer cells via transfer of tumor-associated molecules (“education”) results in the sequestration of circulating mRNA. These tumor-educated platelets could provide a strong indication on tumor type and molecular subclass and be used to broadly scan for molecular traces of tumors.
OTHER BODY FLUIDS AS ctDNA SOURCES

Apart from blood, ctDNA has been detected in several other body fluids, such as urine and cerebrospinal fluid (CSF) (Figure 6), and their analysis alongside blood can provide complementary information21.

1) Urine

Several studies have revealed the presence of ctDNA in urine of bladder cancer patients29,30 (also known as tumor-derived transrenal DNA). However, at present, quantification of this kind of ctDNA is technically challenging, mainly due to its low concentration, although further development of sequencing technologies will probably facilitate this kind of analysis31. Moreover, urine liquid biopsies are truly non-invasive, and the urine sample can be collected by the patients themselves at home.

Figure 5. Different body fluids (apart from blood) are sources of ctDNA. CSF, cerebrospinal fluid. Modified from Siravegna et al.

Figure 4. Tumors "educate" platelets by altering their mRNA profile.
ctDNA can be detected in the blood of the majority of metastatic cancer patients; however, patients with brain tumors have very low levels of ctDNA in blood, even those with high-grade gliomas and medulloblastomas. Although not directly proved, it has been suggested that the blood-brain barrier could prevent the entry of ctDNA into the circulation, and this would explain the low ctDNA concentration in blood. On the other side, the proportion of ctDNA in CSF in brain tumor patients has been shown to be significantly higher, and although lumbar puncture – the procedure to obtain CSF – is an invasive method, it is performed in routine in brain tumor patients. Moreover, evidence suggests that if a tumor metastasizes to the brain, CSF could be used for ctDNA analysis.

### CONCLUSION

Solid biopsies will continue to have a main role in cancer management; however, the use of tissue specimens is limited because repeated biopsy is not practical (invasive protocol) and they may not capture tumor heterogeneity. On the other hand, ctDNA in blood detects tumor genetic alterations in a non-invasive manner – allowing serial sampling for studying treatment response or relapse –, gives an overview about heterogeneity and can predict cancer recurrence earlier than conventional techniques. The fact that New York state recently approved the first blood biopsy test confirms the potential of ctDNA analysis in cancer care.

In addition to blood ctDNA, precious complementary information can be obtained from the ctDNA present in other body fluids or from the mRNA in exosomes or tumor-educated platelets. The integration of all these data will allow the effective molecular characterisation of cancer patients, providing the tools for a personalised medicine.
REFERENCES


