Liquid biopsy: Decoding cancer in blood.

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Abstract

Cancer is that awful tsunami which we all get intimidated to face when we are suspected to be suffering from it and is the last word we expect to hear from the doctor's mouth. The world we live in, suddenly changes and the people we share it with begin to illuminate things we did not even pay attention to. The long standing fight against this so called dreadful tornado in medical science has been based upon improving innovative but less invasive screening programs and methodologies for early detection of cancer. We cannot direct the wind but we can adjust the sails. In other words, there is much less to be done to avoid the onset of this tsunami but a lot can be done to prevent the aftermath. The sooner cancer is diagnosed and treated, the better a person's chance for a full recovery.

Keywords: Liquid biopsy, Decoding, Cancer, Blood

Introduction

Liquid biopsy is a diagnostic repeatable test, which in last years has emerged as a powerful tool for profiling cancer genomes in real-time with minimal invasiveness and tailoring oncological decision-making. It analyzes different blood-circulating biomarkers and circulating tumor DNA (ctDNA) is the preferred one. A wide number of technologies have been developed with the aim of increasing their sensitivity and specificity with acceptable costs. Moreover, several preclinical and clinical studies have been conducted to better understand liquid biopsy clinical utility.

Conventional methodologies for diagnosis of cancer

Traditionally, biopsy methods that often involve a surgeon's scalpel and anesthesia have remained the gold standard of care for diagnosis of solid tumors. Currently, the routine approach for cancer diagnosis involves the examination of tumor tissue through either removing cells using a small needle (fine needle aspiration cytology or FNAC), or histological examination of a biopsy or surgical excision specimen. In patients with solid tumors, biopsies allow the histological definition of the disease and more recently, analysis of solid tumors obtained from surgical or biopsy specimens and the information gathered from these biopsies about the tumor-linked genetic alterations is increasingly used for diagnostic, prognostic and treatment purposes [1]. Despite these advances, a tissue sample preparation for analysis and diagnosis needs a biopsy, excision or another invasive procedure such as fine needle aspiration. However, there are limitations to routine usage of both these methods owing to their invasive nature and certain clinical complications [2].

For instance, the small tissue amounts from fine-needle aspirates or core-needle biopsies often result in smaller amounts of tumor tissue for molecular analysis in comparison with surgically resected specimens. While, the tissue biopsy provides a spatial and temporally restricted, single snap-shot information of the tumor under investigation resulting in failing to reflect the true heterogeneity of the tumor which characterizes most advanced cancers. Cancers are heterogeneous, with different areas of the same tumor showing different genetic profiles (*i.e,* intratumoral heterogeneity); likewise, heterogeneity exists between metastases within the same patient (*i.e,* inter-metastatic heterogeneity). A biopsy or tissue section from one part of a solitary tumor will miss the molecular intratumoral as well as inter-metastatic heterogeneity [3].

Furthermore, the majority of tissue samples used for diagnosis are Formalin Fixed, Paraffin Embedded samples (FFPE) which end up in a number of chemical reactions including DNA denaturation, fragmentation of DNA, introduction of non-reproducible sequence alterations apart from cross-linking the tumor DNA and RNA making them difficult for molecular testing and cancer sequencing studies. In this scenario, taking multiple biopsies from the patients primary tumor and metastases would seem to be an answer but due to complexity of procuring the tissue sample which includes the discomfort suffered by the patient, inherent clinical risks to the patient, potential surgical complications like inaccessible tumors leading to cancer seeding/invading to neighboring sites and economic considerations the multiple or serial biopsies options are often impractical. These restraints hinder the ability of a clinician to detect putative therapeutic biomarkers at an early stage towards a successful and fruitful change in cancer treatment before therapeutic resistance (tumors acquire new mutations that render them resistant to the therapies that target specific genetic mutations) creeps in [4].

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Literature Review

Advent of liquid biopsy: Genotyping tumor DNA and tumor cells in circulation

The need of the hour is thus to devise rapid, cost-effective and non-invasive, patient-friendly techniques which can aid in the monitoring of tumor genomes and identify potential biomarkers to track molecular changes cancer cells undergo upon exposure to therapy. Hence, to reckon with the limitations on the use of single biopsies, new ways to monitor tumor genetics and tumor dynamics have evolved [5]. At the outset, scientists Mandel and Metais in 1948, first reported finding circulating free DNA (cfDNA) and RNA in human blood, without realizing its clinical utility at that time. Ironically, the first practical use of circulating DNA came in another field, Dennis Lo, a chemical pathologist, successfully showed that pregnant women carrying male babies had fetal Y chromosomes in their blood. This finding paved the way for doctors to investigate fetal DNA in circulation of expecting mothers to reveal germline fetal changes weeks after conception, including point mutation, aneuploidy and developmental disorders such as down's syndrome without resorting to invasive testing and has revolutionized the field of prenatal diagnostics [6].

Astoundingly, the levels of cfDNA were higher in diseased than healthy individuals, indicating that it is possible to screen for the presence of cancer through a simple blood test. To add on, it took 17 more years to show that this DNA had mutations which were hallmarks of cancer, a proof that it originated from tumors and this DNA was termed in oncology as circulating tumor DNA (ct DNA) [7]. Very recently, researchers have found cancer cells upon their rupture through apoptosis and death by necrosis, release or sometimes even secrete their contents, including circulating tumour DNA (ctDNA)/genome fragments in circulation that float freely through the bloodstream. Debris from normal cells is normally mopped up and destroyed by ‘cleaning cells’ such as infiltrating macro phages, but tumors are so large and their cells multiply so quickly that the cleaners cannot cope completely. Cancer cells released by cell detachment from primary tumor mass into the bloodstream are known as Circulating Tumor Cells (CTCs). Detected in the blood of patients with solid tumors, including breast, prostate, lung and colon, CTC enumeration serves as a marker for tumor growth as well as for defining tumor aggressiveness [8].

As CTCs and ctDNAs are potential surrogates for the tumor itself and can in principle provide the same genetic information as the tissue biopsy, with more techniques developed/refined for measuring and sequencing tumor DNA (ctDNA) and also for measuring CTCs in the bloodstream, scientists are now turning vials of blood into liquid biopsies [9]. Taken over time, testing such blood samples with CTCs and ctDNA serving as more potential and sensitive biomarkers, would reveal to clinicians whether treatments are working and whether tumors are evolving resistance thus serving as ways to get a richer view of a patient’s cancer and even track it over time. For e.g. though most protein biomarkers stay in the blood for weeks, ctDNA with a half-life of less than two hours renders a clearer view of a tumor’s present status rather than its past changes [10]. Two research teams at Cambridge and John Hopkins have found that ctDNA is a more sensitive biomarker than the protein biomarkers for detection of breast and bowel cancers respectively and also exhibits higher accuracy at tracking tumor disappearance, spread and recurrence. Therefore, there are clear advantages in measuring ctDNA as a marker of tumor dynamics over conventional protein biomarkers or even imaging studies [11].

Discussion

Technological advances in detection of circulating tumor DNA

However, detection of cfDNA derived from tumors, also known as circulating tumor DNA (ctDNA), has been challenging for three primary reasons, which include: Discrimination of ctDNA from normal cfDNA; presence of sometimes extremely low levels of ctDNA; and the accurate quantification of the number of mutant fragments in a sample. Discriminating ctDNA from normal cfDNA is aided by the fact that tumor DNA is defined by the presence of somatic mutations, usually single base-pair substitutions like point mutations (EGFR and KRAS), rearrangements (EML4-ALK), amplifications (HER2 and MET) and even aneuploidy which typify the genome of cancer cells or precancerous cells and are not present in the DNA of normal cells of the same individual, thereby assuring ctDNA an exquisite biologic specificity as a biomarker. In people with very advanced cancers, tumors might be the source of most of the circulating DNA in the blood, but more commonly, ctDNA makes up barely 1% of the total and possibly as little as 0.01%. Advances in technology in the past decade, have resulted in the development of new techniques which allow for enumeration of rare mutant variants in complex mixtures of DNA in blood, plasma or serum and include novel sequencing technologies like Pyrophosphorolysis Activated Polymerization (PAP), Tagged-Amplicon Deep Sequencing (TAM-Seq), massive parallel sequencing, highly sensitive quantitative Polymerase Chain Reaction (PCR) testing and BEAMing (Beads, Emulsions, Amplification and Magnetics) digital PCR.

For example, an amplification method known as BEAMing developed by genetic oncologists, Bert Vogelstein and Kenneth Kinzler at Johns Hopkins which fastens circulating DNA to magnetic beads that can then be isolated and counted can detect ctDNA even if it is outnumbered by healthy cell DNA by a factor of 10,000 to 1. The oncologists utilized it to track ctDNA in 18 people who were being treated for bowel cancer. After surgery, the patients ctDNA levels fell by 99%, but in many cases the signal did not disappear completely and in all the patients displaying detectable ctDNA at the first follow-up appointment, the tumors eventually returned except in one patient and strikingly none of the patients with undetectable levels after surgery experienced a recurrence.

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In another instance, Mary Susan Sabini, a fifth-grade teacher from Gardiner, New York, had lung cancer that resisted two attempts at chemotherapy and a round of radiation. Her doctors at Sloan Kettering Cancer Centre saw ctDNA in her blood when she began taking an experimental drug it was her last hope. Four days later, the cancer DNA shards had vanished, a sign, the doctors hoped, that the treatment was working. Within weeks, Ms. Sabini began to breathe easier. Months later, she had a CT scan, an x-ray test that confirmed her tumors were shrinking. "Every cancer has a mutation that can be followed with this method," said Dr. David Hyman, the oncologist at Sloan Kettering who is leading the study of the experimental drug Ms. Sabini takes "liquid biopsy is like bar coding the cancer in the blood." So on and so forth, researchers and medical oncologists around the globe are finding out things about individuals cancers that astonish them. These instances suggested that ctDNA levels in blood or liquid biopsy can reveal how well a patient has responded to surgery and whether they need chemotherapy to finish off any lingering cancer cells [12].

Liquid biopsy as a game changer in cancer diagnosis and treatment

Liquid biopsy has thus served as a game changer in cancer diagnosis and therapy owing to the following advantageous applications can be captured and characterized for biomarkers similar to tissue; allows early disease detection; allows evaluation of metastasis in real-time and monitoring of the actual treatment response; enables investigation of primary tumors and metastases through simple, non-invasive blood tests with clear advantages such as being a source of fresh DNA, sample is unhampered by preservatives; enables assessment of tumor heterogeneity and monitoring of tumor dynamics as blood can be drawn at any time during the course of therapy and allow for dynamic monitoring of molecular changes in the tumor rather than relying on a static time point for resecting a solid tumor for tissue biopsy; enables study of the "tumor dormancy" phenomenon and is much faster and cost effective than classical biopsy testing.

Open challenges

In theory, the use of liquid biopsies to identify early relapse or progression is very attractive, but there are still some open issues still some unknowns, too. Does ctDNA paint a truly representative portrait of every cancer? Do tumors that have spread to other organs release as much DNA as the original tumors? Do all the cells in a tumor release as much ctDNA as each other after death and compare them with ctDNA extracted in life. And the biggest question remains: Does an accurate picture of tumor burden or a real-time look at emerging mutations, actually save patients or improve their quality of life? Even though a tumor has developed a resistance mutation, that insight is useless if there are no drugs that target the mutation.

Conclusion

Nevertheless, it is exciting to imagine that liquid biopsy still remains a simple blood draw that could tell doctors what they need to know to treat cancer more effectively, although there are many obstacles that must be overcome before we will see "liquid biopsies" joining the ranks of standard medical tests. But, it is the modern era's best and most promising answer to the world's most dangerous tsunami in medical science "cancer". In other words, there is much less to be done to avoid the onset of this tsunami but a lot can be done to prevent the aftermath. The sooner cancer is diagnosed and treated, the better a person's chance for a full recovery.

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