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Original Research Paper

Oncology

WHY LIQUID BIOPSY MAY PROVE TO BE GAME CHANGER IN COMING DAYS FOR ONCOLOGY TREATMENT AND IT'S DIAGNOSIS AS WELL AS ITS TREATMENT PREDICTION !

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ABSTRACT

Over the last decades, the concept of precision medicine has dramatically renewed the field of medical oncology; the introduction of patient-tailored therapies has significantly improved all measurable outcomes. Liquid biopsy is a revolutionary technique that is opening previously unexpected perspectives. It consists of the detection and isolation of circulating tumor cells, circulating tumor DNA and exosomes, as a source of genomic and proteomic information in patients with cancer. Many technical hurdles have been resolved thanks to newly developed techniques and next-generation sequencing analyses, allowing a broad application of liquid biopsy in a wide range of settings. Initially correlated to prognosis, liquid biopsy data are now being studied for cancer diagnosis, hopefully including screenings, and most importantly for the prediction of response or resistance to given treatments. In particular, the identification of specific mutations in target genes can aid in therapeutic decisions, both in the appropriateness of treatment and in the advanced identification of secondary resistance, aiming to early diagnose disease progression.

KEYWORDS : Liquid biopsy, Circulating Tumor Cells, circulating tumor DNA, Exosomes

DISCUSSION

Traditional biopsies and surgical procedures are invasive, charged with potential complications, sometimes unrepeatable and cannot be performed when clinical conditions have worsened or when a tumor is inaccessible. Furthermore, the genomic profile of biopsy tissues provides a tumor picture limited to a single point in time, and may also show the genetic heterogeneity of numerous tumor subclones. In fact, many studies have established that the genomic landscape of tumors and metastases dynamically evolve over time in response to selective pressure of therapies that can suppress or promote the growth of different cellular clones. These limitations are particularly evident in the presence of acquired resistance to therapy or in monitoring the disease during follow up. For these reasons, in recent years the new field of oncology research has focused on cancer-derived components that circulate in the bloodstream. Apoptotic or necrotic cancer cells release circulating cellfree DNA fragments, designated as circulating tumor DNA (ctDNA), as well as exosomes (EXOs), namely membrane-ncapsulated subcellular structures containing proteins and nucleic acids released by the tumor cells.

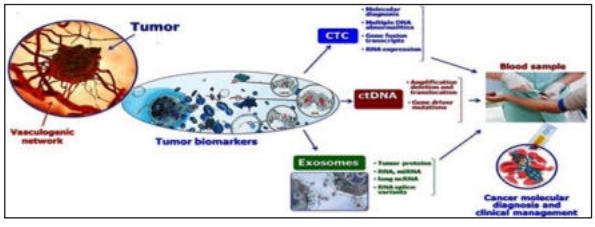

Fig : 1 In molecular oncology ,circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) and exosomes use as tool for liquid biopsy

Primary tumor and metastatic sites are also able to exfoliate vital cells that, once entered into the bloodstream, are circulating tumor cells (CTCs).Isolation of these tumorderived components from peripheral blood and their genomic or proteomic assessment represent a new diagnostic tool that has been called 'liquid biopsy' .The initial limitations due to the scarcity of nucleic acid as well as the difficulty in distinguishing between normal and tumoral nucleic acids have been overcome by the increased sensitivity of next-generation sequencing (NGS) techniques, which now may accurately detect genetic and epigenetic aberrations.

Circulating Tumor Cells Its And Detection Techniques

. CTCs are shed from either primary or secondary tumor sites; they migrate into the circulatory system and are responsible for the development of distant metastases. CTCs are extremely rare, occurring at a frequency as low as 1 CTC per 106–107 leukocytes, with even lower numbers in early stage diseases. Initially assessed as non leukocytic, nucleated cells of epithelial origin, CTCs do not have well defined morphological aspects and they may vary according to cancer type and stage. CTCs may also cluster either with parental tumor cells or with fibroblasts, leukocytes, endothelial cells or platelets, forming aggregates with higher propensity to seed distant metastases than single CTCs, thanks to a their survival advantage and to the protection from the immune system and oxidative stress. However, CTCs provide an ideal approach to molecular cancer diagnosis and treatment options, and their investigation is widespread in cancer research. The major differences are their large size, up to 20–30 □m, mechanical plasticity, and dielectric mobility properties compared with blood cells. Fruitful methods of isolation include membrane filtration, density gradient stratification, dielectric mobility, photoacustic and microfluidic separation.

To date, the CellSearch (Menarini Silicon Biosystems, Firenze, Italy) assay, using the antibody-based immunomagnetic technique and image cytometry, is the only US Food and Drug Administration (FDA) approved CTC diagnostic technology for metastatic breast, prostate, and colorectal cancer, whereas the recent DEPArray system allows both detection and recovery of single CTCs by surface or cytoplasmic markers, as well as size and dielectrophoretic movimentation

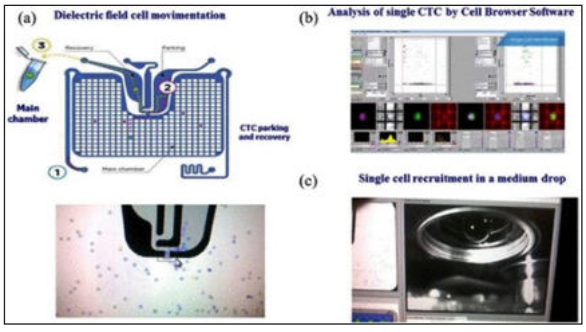

Fig-2 -The DEPArray technology is based on the use of a dielectrophoretic field (DEP) generated by electrodes in a matrix underlying a liquid layer of cells. (a) The DEPArray constellation for the creation of DEP is determined to be a determinant of the entire intrapolarization. (b) The whole cell is isolated from the individual software, and (c) after

computational imaging, individual cells or groups of cells are moved and recovered by a drop of buffer in a specific tube

Clinical Application Of Ctdna

As described, ctDNA may be used as a biomarker with several clinical applications in solid tumors. It is comparable to a maneuverable, easy to find, always updated snapshot of the tumor, capable of reflecting its dimension, molecular heterogeneity, and its evolution over time. Accordingly, its potential applications are numerous, starting from cancer screening. Beck and colleagues investigated the profile of circulating DNA in healthy subjects and found that ctDNA provides useful baseline information regarding the subclinical conditions of patients, including cases of unknown neoplasms that correlate with specific mutations, loss of heterozygosity, or methylation patterns. Subsequently, others assessed the correlation between cancer-related DNA and the development of tumors, However, the results were controversial since ctDNA and cancer-related mutations are also detectable in apparently healthy individuals several years before the clinical evidence of cancer development,but the same mutations in ctDNA can be detected in healthy volunteers who will never develop a cancer.Therefore, performing screening tests based on ctDNA in the asymptomatic population could cause overdiagnosis, but the screening of subjects with known risk factors for developing cancer could overtake this problem. NA can be used to stratify patients at variable risk of recurrence after surgery, selecting who can really benefit from an adjuvant treatment and avoid unnecessary therapies and their relative systemic toxicities. Furthermore, the molecular properties of ctDNA may address treatment options. The characteristics of ctDNA are the mirror of the tumor's molecular profile. In patients with breast cancer,mutations of TP53 were found both in ctDNA and tumor tissue with a concordance of 43%,. Treatment with targeted therapies puts the tumor cells under a selective pressure, thus allowing a clonal evolution in a Darwinian manner. Therefore, prolonged treatment with targeted therapies is capable of selecting the cell clone resistant to therapy.. The correlation between KRAS mutations in patients with colorectal cancer and the response to therapy was first investigated by Misale and colleagues, who demonstrated that there is a gradual increase in KRAS-mutated ctDNA in a patient's serum during treatment with panitumumab or cetuximab. It is therefore conceivable that KRAS mutant clones, present in the primary lesion, undergo a pressure selection that allows the growth of mutant cells.

Exosomes isolation and characterization

EXOs are nano-sized vesicles (40–100 nm) released by cells and detectable in most body fluids, such as plasma, urine, saliva, or ascites., EXOs are end products of the recycling endosomal pathway and originate from inward budding of the plasma membrane. Although they were previously considered as cellular waste products.

EXOs take part in many physiological and pathological processes and have been shown to be involved in cancer progression and metastatization. Noteworthy, EXOs released by cancer cells, namely tumorderived EXOs, promote EMT and affect the proliferation, migration, and invasion of cancer cells, as well as support the angiogenesis and the establishment of an immunosuppressive milieu. EXOs are also emerging as a novel chemoresistance mechanism, primarily depending on drug discharge *via* vesicle budding, neutralization of antibody-based drugs, and EXOmediated transfer of micro RNAs (miRNA).

EXOs consist of a lipid bilayer which contains both transmembrane and nonmembrane proteins, as well as noncoding RNAs, mRNAs, and either single-stranded or double-stranded DNA. According to proteomic analyses,

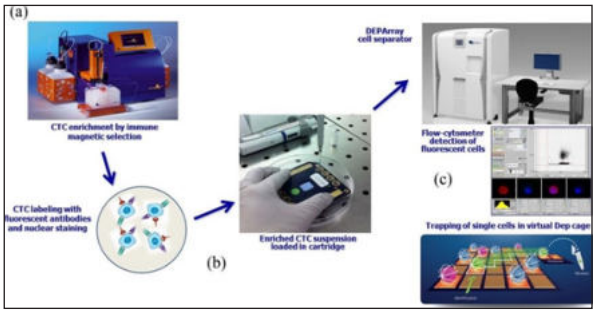

Fig:3- Several methods have been developed to efficiently collect EXOs from body fluids

Methods to isolate circulating tumor cells (CTCs).

a. Peripheral blood samples are subjected to density gradient stratification and leukocyte depletion is assessed by an immunomagnetic method
b. The CTC-enriched fraction is stained by specific fluorochrome conjugated antibodies and loaded in a dedicated cartridge which then is subjected to dielectric forces
c. CTCs are visualized by dedicated software and selected by positive fluorescence for tumor-specific markers and negativity for CD45 leukocyte marker.4',6-diaminidino-2phenylindole (DAPI) is used to counter stain nuclei. The CTCs are moved into a parking area and recovered as single or grouped cells.

REFERENCES

1. McCarthy JJ, McLeod HL and Ginsburg GS. Genomic medicine: a decade of successes, challenges, and opportunities. Sci Transl Med 2013; 5: 189sr4.
2. Hodson R. Precision medicine. Nature 2016; 537: S49.
3. Savonarola A, Palmirotta R, Guadagni F and Silvestris F. Pharmacogenetics and pharmacogenomics: role of mutational analysis in anti-cancer targeted therapy. Pharmacogenomics J 2012; 12: 277–286.
4. Wang J, Chang S, Li G and Sun Y. Application of liquid biopsy in precision medicine: opportunities and challenges. Front Med 2017; 11: 522–527.
5. Perakis S and Speicher MR. Emerging concepts in liquid biopsies. BMC Med 2017; 15: 75.
6. Siravegna G, Marsoni S, Siena S and Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol 2017; 14: 531–548.
7. Crowley E, Di Nicolantonio F, Loupakis F and Bardelli A. Liquid biopsy: monitoring cancergenetics in the blood. Nat Rev Clin Oncol 2013; 10: 472–484.
8. Buder A, Tomuta C and Filipits M. The potential of liquid biopsies. Curr Opin Oncol 2016; 28: 130–134.
9. Zhang W, Xia W, Lv Z, Ni C, Xin Y and Yang L. Liquid Biopsy for Cancer: Circulating Tumor Cells, Circulating Free DNA or Exosomes? Cell Physiol Biochem2017; 41: 755–768.