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Review Article

LIQUID PROFILING: A NOVEL PANEL FOR CANCER DETECTION, CURE AND THERAPEUTIC RESISTANCE

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ABSTRACT

In future tumor DNA and tumor cells in circulating fluids plays crucial cancer marker in field of cancer diagnosis and therapy. The genotyping of somatic genetic alterations reveals more informative about tumor progress but has limitations. A liquid profile of circulating nucleic acids is a less painful procedure substitute with surgical biopsies of solid tissues, usually obtained through the extraction of a blood sample or other body fluids. Cell free genetic fragments are released into the blood circulation by all nucleated cells through process apoptosis or necrosis, and amount of circulating cell-free DNA (cfDNA) correlates with various levels of tumor and its prognosis. Fortunately ctDNA frequently signifies a small fraction (1.0%) of total cfDNA. ctDNA differentiated from normal DNA in presence of altered sequence. Moreover the ctDNAs identification shows mainly tumor-related genetic and epigenetic variations. Circulating tumor cells (CTC) in blood sample makes us to known number of cancer related phenomenon, but not limited to the presence of mutations, loss of heterozygosity, gene over expression, alternative splicing, drug sensitivity and resistance. This review illustrate about circulating tumor DNA and tumor cells in diagnostic perspective and presents on existing challenges of cancer diagnosis, cure, and drug resistance.

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INTRODUCTION

The malignant cancer transmission to other regions finds out the main cause of death for cancer patients up to 90% of the cases [1, 2]. Cancer can be identified and diagnosed based up medical imaging, immunohistology and molecular analysis. In tumor growth and its advance stages , the clinical decisions are usually made based on the reports of diagnostic routine analysis of primary tumor specimens and characteristic signs of disease , but genetic and epigenetic map shows evolutionary changes may occur [3,4]. Circulating fluids are mainly blood, saliva, urine and cerebrospinal fluid. Most frequently assessed circulating fluid is serum which consists of fragmented DNA which is cell-free component and clinical usages of circulating cell free DNA (cfDNA) in plasma and serum, mainly presented by mendal and Metais in 1948[5]. Evaluation of fetal DNA in blood after couple of weeks can reveal germ line fetal changes, including aneuploidy and point mutations, and this is potentially turned into standard care in prenatal diagnosis in critical ill patients [6, 7].

The term liquid profiling or liquid biopsy includes circulating tumor DNA (ctDNA/cell-free DNA (cfDNA), circulating tumor cells (CTCs), circulating RNAs and exosomes[8, 9]. The total cell-free DNA (cfDNA) in blood circulation of patients is mainly derived from tissue physiological remodeling events [10]. Usually the measurable percentage of ctDNA in blood is between 0.1-10 of the total cfDNA[11]

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In recent time's ctDNA, Circulating tumor cells and exosomes have increased awareness of tumor-related material that can be found in body fluids of patients [12]. Circulating tumor cells are released mainly from primary tumor and utilize blood and lymph system as main way to travel other parts and develop into metastatic tumors. The tumor of patient consists of exosomal proteins, RNA and DNA and used as investigative tool in cancer healing, resistance, accurate treatment and prediction [12]. Circulating RNAs includes coding RNAs, non coding RNAs and MiRNAs. This review mainly explains about advances of CTCs and ctDNA in liquid profiling for cancer management.

Circulating tumor cells as marker

Tumor cells in circulation were first narrated in year 1869 [13], later its progress attained through heterogeneous blood components for various constant biotechnological applications. Circulating tumor cells (CTC) which are derived from metastatic lesion or primary tumor at one site reveal metastatic lesion at distant site. This primary tumor and metastatic lesion release vital cells in to circulation which are called as circulating tumor cells. Recently, advanced CTCs techniques has been developed in which CellSearch System is the only technique approved by US Food Drug Admistration (FDA) [14], though its sister technique has been emerged and named as antigen based CTC[15]. CellSearch System is the two step technique; in which first step consist of centrifugation of sample to remove plasma components whereas CTCs are caught with anti-epithelial adhesion molecule (Ep-CAM)-conjugated magnetic ferrofluids [15]. In the second step, unrecognized CTCs are stained and recognized by using anti-cyto keratin antibodies. Recent studies suggest that CTCs matched bone marrow-derived tumor cells shows high concordance in somatic a mutation, a classical example is multiple myeloma [16-18]. In one of the study, CTCs panel was designed to investigate early diagnosis of hepatocellular carcinoma and sort out differential diagnosis from liver cirrhosis, chronic hepatits B infection related carcinoma and benign hepatic lesions [19]. Study conducted by jang ho cho et al shows enriched CTCs isolated from CSF fluid through analysis of DEPArray technology with leptomeningeal seeding from advanced gastric cancer demonstrates intra-patient heterogeneity at the single cell level[20]. The probability of patient response to the treatment and prior to the treatment done (i.e., patient stratification) is accomplished by CTCs analysis. In small cell-cell lung cancer, detection of a CNVs-classifier to establish Chemosenstive versus chemorefractory individuals and copy number variations sequenced in CTCs [21]. This CTCs-based CNV classifier can able to give out 83% of tested patients as chemorefractory or chemosenstive with help of pretreated blood samples. It is important to note the study findings shows patients who are chemosenstive initially eventually developed resistance but chemorefractory patients did not acquire the same CNV Profile, this indicate that the genetic basis of acquired resistance and innate may vary[21].In other study, Melanoma RNA expressed from CTCs profile before giving immune checkpoint inhibitors therapy [22].

Circulating ctDNA as marker

In laboratory medicine practice, plasma extracted ctDNA is the most frequently used form of blood-based biomarker compared to CTCs. In cancer patients, to overcome the inherent risks related to tissue biopsies, uses of ctDNA well described for disease screening [23]. The DNA fragments derived from cancer cells released in blood circulation consist of tumor-specific genetic information or epigenetic alterations which are not seen normal cfDNA [24]. Recently, data gathered from various Asian cohort studies shows ctDNA in blood circulation correlates with the altered gene sequence of tumor tissue extracted from hepatocellular carcinoma [24]. Presence of epigenetic in tumor DNA discriminates from normal cfDNA. The genomes of precancerous and cancer cells are differed from normal cells by presence of somatic mutations most frequently single base-pair substitutions [25]. This combination states exquisite ctDNA as tumor marker and have biological specificity. Therefore, DNA sequencing used in somatic mutations analysis used similar to identify easily ctDNA in cancer patients when it is abundantly present in circulation [25]. Patients with serious tumor burden and elevated levels of ctDNA which correlates with mutant fragments can be detected by standard sequencing approaches like Sanger sequencing or pyro sequencing [25]. Digital genomic approaches have high sensitivity in identification of the altered sequence of ctDNA fraction matching to advanced tumor tissue in practically every case [26-28]. Detection of ctDNA in aneuploidy, rearrangements and amplification with significant high sensitivity shows effective analytical methods (shown fig 1). ctDNA analysis has been extensively used for identification of actionable mutations for example the detection of EGFR mutations in non-small lung cancers [29] and its use in early cancer identification is still under research [30], Furthermore, study conducted by Adalsteinsson VA et al [31] by using statistical software (ichorCNA), the ctDNA content in cfDNA samples assessed to full fill the criteria of exon sequencing. In study conducted by Corcoran RB et al [32] shows ctDNA used to identify early type of cancers in asymptomatic patient. The clinical applications of ctDNA as follows (1) detection of altered gene sequence of interest include mutations which cause resistance to therapy, in ctDNA. (2) Early identification of recurrence of cancer in minimal residual recurrence. (3) Early detection of primary source of disease. (4) Detection of

genetic determinants for effective therapy. (5) Reveals metabolic study of tumors. In medical practice, in order to use ctDNA as biomarker, following requirements should be fulfilled [33]: analytical test should be accurate, precise and reliable, high analytical validated, standard prognostic factors combined with validated predictive /prognostic markers.

Sample collection, preservation and methods for cfDNA analysis

Body fluids like saliva, ascities, pleural effusion, cerebrospinal fluid (CSF), urine or stool can be used for cfDNA analysis. Most studies suggest plasma is suitable sample for cfDNA analysis, because in collection of serum, it is released in test tubes from red blood cell lysis during clotting process before centrifugation, which contaminates and shows higher levels [34]. Peripheral blood is the most common source for the extraction of cfDNA. Extraction cfDNA to be done within 4-5 hrs after withdrawn and minimum amount of blood required is 1 ml. There are many methods for detection of single gene mutations (such as epidermal growth factor receptor mutations (EGFR), among all these, BEAMing digital PCR method is most sensitive method reaching approximately 0.01% sensitivity in comparison with other methods as shown in the figure 1. The four important methods used to identify ctDNA EGFR mutations comprise two amplification refractory mutation systems (ADx-ARMS and cobas-ARMS) a droplet digital polymerization reaction (ddPCR) and next generation sequencing (Firefly platform), out of these firefly and cobas-ARMS are more sensitive[35].

Table 1. Shows classical difference between tissue biopsy and liquid profiling

Tissue biopsy	Liquid profiling
Painful procedure and uncomfortable May exhibit other clinical conditions	Less painful and convenient procedure Does not exhibit other clinical conditions
Need of specialized surgical skills	Need minimum medical skills and surgical facilities
Need clinical histology laboratory and trained professional	Need specialized laboratory equipment for DNA and RNA isolation and trained technical professional
Does not represent tumor heterogeneity	Representative of tumor heterogeneity
DNA-cross links (FFPE only)/ risk of DNA degradation	Careful collection and processing samples needed Low concentration complicates detection
Cannot be repeated often	Can be repeated when samples stored in prescribed temperature conditions.
Difficult to follow up the patient	Dynamic monitoring of molecular changing

Figure 1 depicts various methods for detecting circulating tumor DNA with sensitivities. Sanger sequencing,[36] amplification refractory mutation system (ARMS),[37,38] Pyro sequencing,[39] pyro phosphorolysis-activated polymerization (PAPS),[40] tagged-amplicon deep sequencing(TAM-seq),[41] digital polymerization chain reaction(PCR),[42] Beads emulsion and amplification and magnetics(BEAMing)[43]

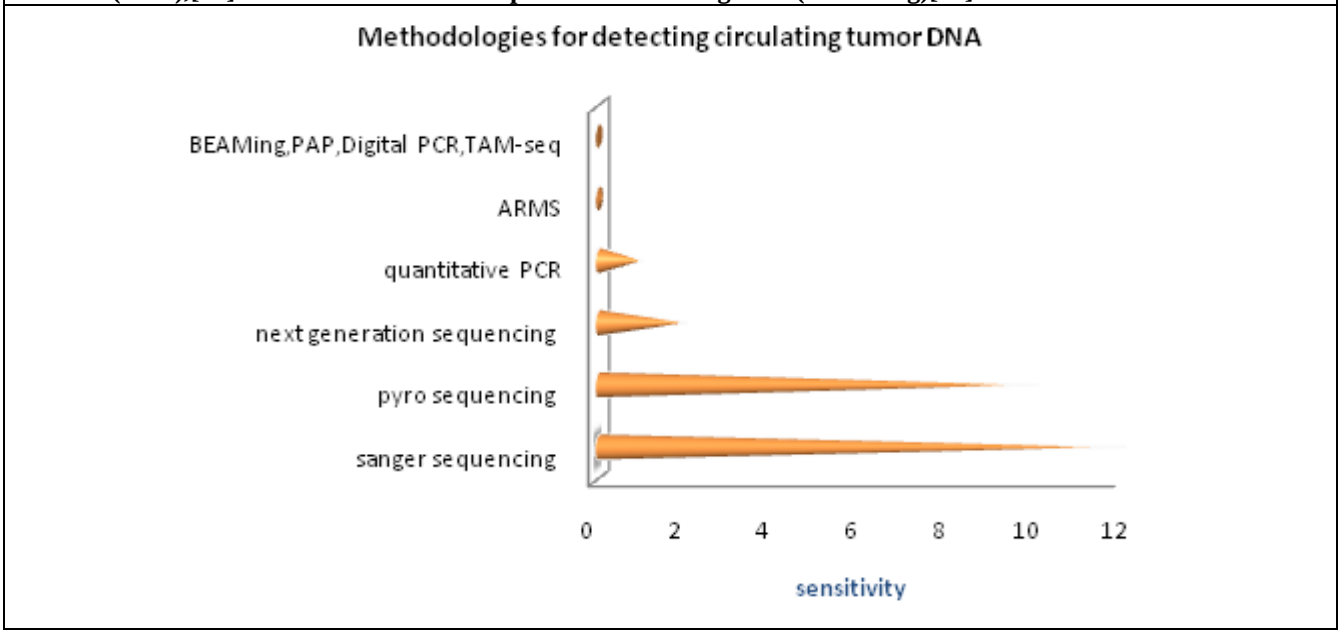


Figure 2. Sketches out association between DNA origin and size. Depending on origin and type of release, circulating DNA can be different in size, ranging from long sized fragments (if release is due to necrosis) to short sized fragments (if release due to apoptosis). Adapted from mouliere et al [50]

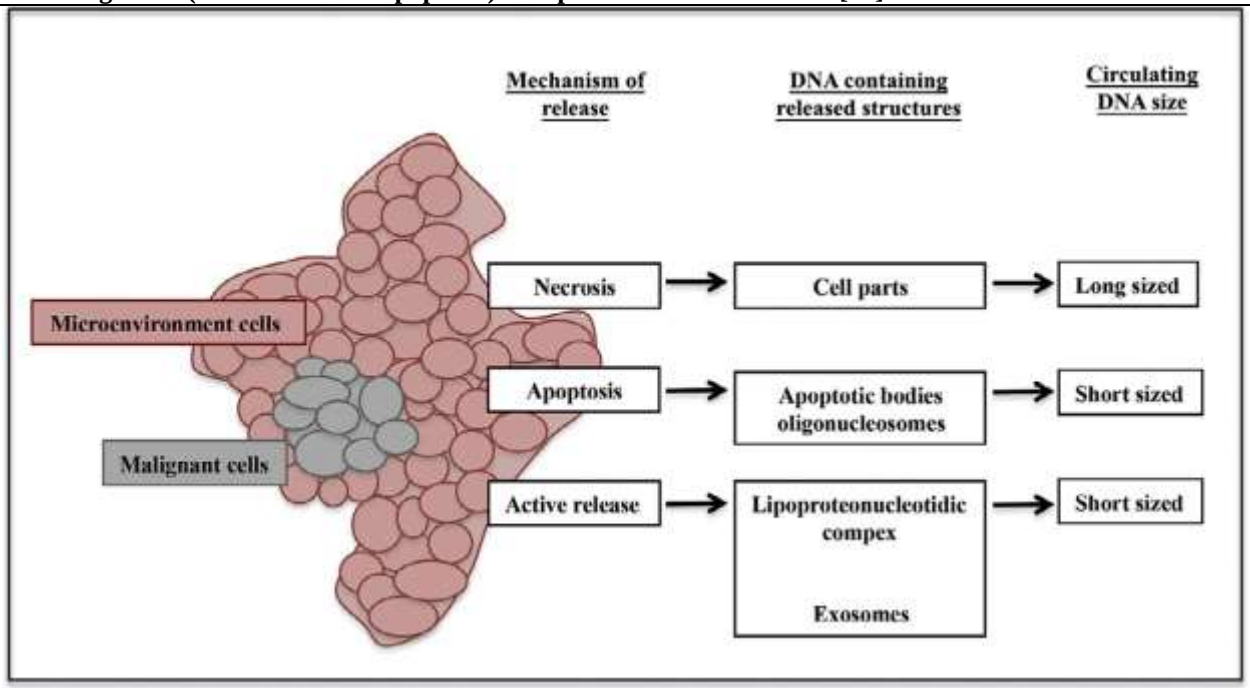


Figure 3. shows genetic changes related to cancer such as point mutations, copy number variation (CNV), chromosomal rearrangements and methylation pattern in circulating cell free DNA. Adapted from Luis A and Diaz et al [25]

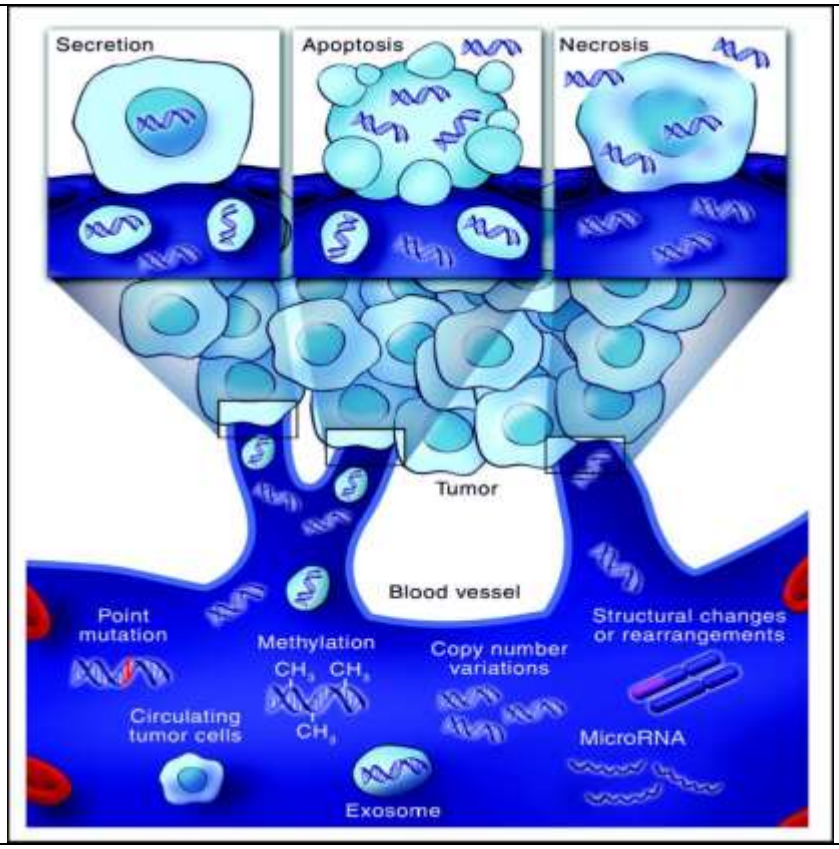
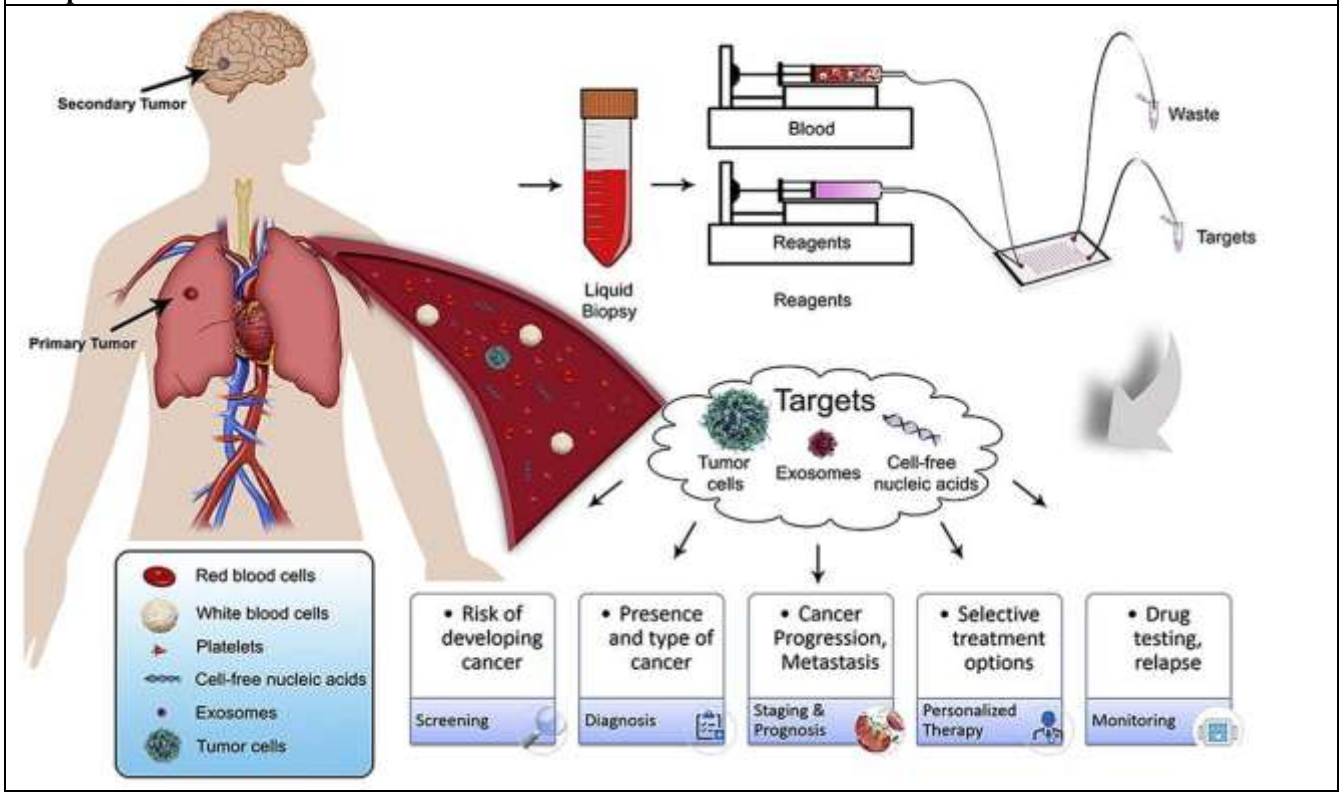


Figure 4. shows various tumor resultant products which can be effectively extracted from Microfluidic techniques. These products with help of different analysis gives valuable information about diagnosis, treatment monitoring. Adapted from Yuxi sun etal104



Mechanism of ctDNA release in to circulation

Plasma or serum of normal healthy people contains minimal amount of circulating DNA. The number of apoptotic and necrotic cells increases as tumor volume increases due to more cell turnover [44, 45]. Under normal physiological phenomenon a small number of number of apoptotic and necrotic cells are formed and scavenged by infiltrating phagocytes. But in cancer patients the phagocytes stops clearing apopotic and necrotic cells due to increase in tumor mass which results in accumulation of cell debris and active DNA discharge into circulation[25]. Released circulating free DNA is double stranded polynucleotide associated with protein complex and its length varies from 18bp to 10000 bp [46]. It is strongly believed that among this cfDNA, the ctDNA is derived from tumor cells are released into circulation along with nucelosomes in single, double, triple forms and mostly fragmentary form. Moreover, half life of ctDNA in the circulation is less than 2 hours after which is cleared from circulation [47].

Studies proved that in patients with cancer have much higher circulating cfDNA compared with healthy individuals [48, 49]. The length of cfDNA strand in circulation reveals characteristic feature of apoptotic process [50] and passive release depends up on location, size, and vascularity of tumor. Interestingly from malignant cells the altered DNA produced differs in size, because not only natural cell death apoptosis occurs but also from necrosis or mitotic catastrophe and autophagy [51] as depicted in figure 2. Most of studies evaluated DNA integrity which signifies the ratio of longer fraction of DNA (necrotic DNA) to shorter ones (apoptotic DNA) [52, 53] depicted in figure 2. One of the important issues about cfDNA in circulation is that mutations present in it are not exactly derived from tumor cells [54]. This review clears about tumor burden directly proportional to length of ctDNA and is anticipated to differ significantly among patients with various clinical histories.

Mechanism of circulating tumor cells release in to circulation

CTCs are discharged from place either primary tumor or secondary tumor in to peripheral blood and accountable for the growth of distant metastasis [55]. CTCs are enormously seldom, occurring at low levels which may be 1CTCs per 10^6-10^7 leukocytes, with even less number in early stage of disease [56]. Initially measured CTCs are non leukocytic, nucleated cells of epithelial origin, which does not have well distinct morphological features and they vary according to cancer stage and type [55]. In easy way to understand tumor cells can transform epithelial-to-mesenchymal transitions

(EMT) and enter into circulation. Mostly CTCs cluster with parental tumor cells or fibroblasts, leukocytes, endothelial cells or platelets and form aggregates with high propagation to form distant metastasis [57]. In contrast with other biomarkers only CTCs make us to indentify accurate cell architectural differences, as well as in vivo studies and functional ex vivo studies [58].

Liquid profile versus tissue Biopsy

Major barriers exist in tumor biopsy acquisition and utility are collection of sample, processing sample and tumor heterogeneity [59] as shown in table 1, though it is the gold standard for clinical decisions and investigation for confirming disease characteristics. In practical point of view, biopsies are not convenient because they are invasive procedure for patients and increase cost of patient care. Most significantly biopsies are useless without clinical symptoms. Significant difference between liquid profile and tissue biopsy is tumor tissue stored in formalin-fixed paraffin-embedded (FFPE) blocks which cross links the DNA and insufficient for sample processing. In each tumor biopsy, the quantity of tumor cells differ based up on the size of specimen obtained and tumor cellularity (percent of tumor). Further in contrast with surgical resected specimens this tumor tissue from core-needle biopsies or needle aspirates is fragmented to small quantity of tumor tissue for molecular sequencing. Heterogeneity is the major limitation of advanced characterized cancers [59, 60]. A mass of tumor tissue under microscopic examination shows different stages of tumor development (primary tumors and metastasis) and different tumor cells has different characteristic features. Cancers are heterogeneous, if the same tumor from different areas showing different genetic sequence called as intratu moral heterogeneous, if tumor shows metastasis different from each other within the same patient called an intermetastatic hetergenous. Molecular intratumoral and inter metastatic heterogeneity of tumors are not viewed by biopsy or tissue sections which can be sequenced with less invasive procedure is by blood sampling from the needle stick [25]. In addition, blood sample drawn at any time during course of cancer treatment allows active monitoring of genetic sequence changes in the tumor rather relying on static point [25].

Identification of various ctDNAs in circulation before and after anticancer treatment gives detailed information about prediction of treatment resistance, assessing efficacy of drug in cancer treatment, monitoring tumor dynamics during treatment, thus allows us accurate therapeutic decisions [61, 62] which may not be possible with tissue biopsy. For example, in cancer patients the use anticancer drugs like gefitinib, eroltinib or combination of pertuzumab and eroltinib and along with this identification T790M discloses about patient tolerance to above drugs [63]. Therefore tissue biopsy may not provide detail information about cancer diagnosis, resistance therapy and clinical decisions on cancer treatment [25]. Therefore liquid profile contains information about genome and its changes related tumor such as copy number variations (CNV), Chromosomal rearrangements, and methylation patterns [24] as shown in figure 3.

Limitations and technical problems associated with ctDNA analysis

In cancer patients those who are on treatment, the most significant function of liquid profile is ability to carry out longitudinal monitoring and find out therapeutic efficacy [64]. In clinical lab the ctDNA measurements are stable and act as potential marker for diagnosis of cancer in clinical setting. One of the technical issues with liquid profile is that both malignant and healthy cells liberate DNA into circulation, which contains only small fraction of ctDNA. In early stages of cancer, low levels of ctDNA are found in circulation which is difficult to identify [65, 66]. Furthermore, it is important to find out accurately ctDNA and differentiate it from cfDNA in patients those are on treatment with radiations and chemotherapy [67]. To make liquid profiling as routine cancer marker or therapeutic marker, there is conspicuous need to standardize methods, such as way of collection of blood samples, technical specifications of measurements, and ctDNA extraction in whole blood samples [68, 69]. Despite of current advances in liquid profiling, analytical sensitivities of ctDNA in circulation has to be improved to find out its small fractions [70]. In addition, during analysis of ctDNA, it is important to regulate sensitivity identification methods to make probability less so that false positive or false negative can be reduced [71].

Prediction of low residual tumors

In many clinical contexts, identification of recurrence in patient who has been recovered from disease after surgery is by exhibiting clinical features and pathological examination [25]. The Tumor, Nodes, Metastasis (TNM) staging which is widely used in stratifying risk of recurrence identifies cell age and aggressiveness of tumor, but no role in determining residual disease [25].

The detection and quantification of ctDNA is a potential marker in detecting minimal residual disease after surgery or other curative therapy, and can predict risk of recurrence of disease. In cancer patients, ctDNA should be measured 6 to 8 weeks after post surgery and before starting adjuvant therapy to facilitate best therapeutic decision [25]. In resectable colorectal cancer patients before operation identification of high amount of ctDNA correlates with disease recurrence and poor prognosis [72]. Thus ctDNA analysis in circulation provides valuable approach for early stage detection of cancers which is useful for screening and management of

cancer patients those who are on treatment [72]. However, more scientific investigators have been focused on post operative samples. Research in colorectal cancer [73, 77], pancreatic cancer [74, 75], and ovarian cancer [76] have specified that ctDNA analysis after surgery helps to find out clinical relapse and poor outcome. Furthermore, study conducted on advanced rectal cancer patients, illustrates that analysis of ctDNA prior to any treatment is not used for identification of disease recurrence [78]. ctDNA is measured to be better than CT scan [74,75] and protein tumor biomarkers [73,77] for identification of relapse because it has positive predictive value and can be analyzed 6.5 months earlier in condition of pancreatic and adenocarcinoma [74, 75].

Utility of ctDNA in evaluation of drug efficacy

In cancer patients, ctDNA identification permits the early measurement of drug response, especially detection of treatment resistance, thus helping physicians to get better treatment plan in a timely manner to reduce drug toxicity and accomplish improved drug efficacy [79]. In lung cancer patients those who are on chemotherapy, drug effectiveness can be evaluated by measurement of the ctDNA with drug-sensitive tumor-specific mutations are found to be less; otherwise, ctDNA levels are high in case of drug resistance. Mutations in ctDNA transiently increases in patients after epidermal growth factor receptor tyrosinase kinase inhibitor (EGFR-TRI) therapy, this phenomenon is noticed in tumors with a small number of dead cells and in chemotherapy patients with high number tumor cells and ctDNA [80]. In prostate cancer patients, treatment with drugs such as olaparib and talazoparib leads to BRCA2 reversion mutations which is significantly associated with resistance of inhibitor of DNA repair protein called as poly (ADP)-ribose polymerase (PARPi) [81]. In III phase of clinical trials in treatment of breast cancer patients with CDK4/6 inhibitors allows identification of changes in ctDNA PIK3CA levels predicts progression-free survival (PFS) after therapy [82]. Estimation of the plasma androgen receptor (AR) gene status predicts resistance to conventional prostate cancer drugs and deteriorated outcome given by anti prostate cancer drugs such as enzalutamide and abiraterone [83-85]. A research conducted by collecting samples of whole-exome sequencing of cfDNA during clinical trials phase II of PARPi olaparib in metastatic prostate cancer established that cfDNA estimation have significant role in identification of somatic mutations and new mutations which shows development of disease [86]. Moreover, sequencing ESRI mutations in ctDNA helps to find out patients who are resistant to next aromatase inhibitor therapy, though they often appear during the therapy of metastatic lesions which concludes that apparent metastatic cancers and metastatic cancers have diverse mechanisms of resistance for desired effective therapies [87]. A study by analyzing multiple recurrent point mutations of ctDNA fibroblast growth factor receptor (FGFR2) kinase domain during the development of acquired anti-BGJ398 intrahepatic cholangiocarcinoma (ICC) shows identification of resistance towards usage of FGFR inhibitor BGJ398 [88]. Additionally, regorafenib gives clinical benefit in patients based on protein biomarker concentration and mutational status which indicates that in real time the analysis of circulating DNA provides practical approach for noninvasive analysis of the tumor genotyping [89].

ctDNA levels correlates with tumor metastasis

In medical practice, the identification of tumors should be done concurrently with the diagnosis primary tumors, lymph node metastasis and distant metastasis. The tumors are categorized in to various stages and each stage allocated with effective therapeutic plan. In current medical practice, tumor staging before surgery is done by CT scan and tissue biopsy. Updated studies shows that estimation of circulating levels of ctDNA in cancer patient may be helpful in understanding the stage of metastasis. The circulating ctDNA levels in patients with early and late stage tumors may be associated with various factors, such as tumor volume [90-92] associated with necrosis, ki67, lymph node metastasis, hematogenous metastasis, allele frequency and EpCAM positive CTC mutations [93]. Interestingly, findings of ctDNA can reveal different characteristic features of metastatic lesions [94]. Research conducted in bladder cancer patient's shows high levels FGFR3 and PIK3CA mutations in ctDNA of blood and urine reflects tumor metastasis [95]. A retrospective research conducted in cancer patients reveals early detection of metastasis (before 11 months) by comparing recent surveillance methods of ctDNA analysis [96]. Additionally, the driver DNA changes in ctDNA and closely resembled metastatic tissue biopsies was invented in mCRPC. This study results suggest that ctDNA estimations can be practically used to genetically stratify patients and monitor prognosis [97]. Estimations of plasma DNA methylation facilitate early identification of metastasis to consider as primary cancer and its propagation to other organs[98]. Recent research studies demonstrated that methylated gene panel measurements shows remarkable results in metastatic breast cancer [99], non small cell lung cancer [100], ovarian cancer [101] and lung cancer [102]. Additionally, research conducted on metastatic colorectal cancer analysis of methylated biomarkers in cfDNA could accomplish better accurate results compared with combined study with next generation sequencing technique (NGS) [103]. In future recent advances like microfluidic technologies plays key role in separation of cellular and molecular information present in blood for finding out tumor metastasis, cancer diagnosis, accurate treatment and drug resistance

monitoring [104], outline picture of various uses of microfluidic technology as depicted in figure 4.

Circulating mtDNA and metabolism in tumor

Tumor cells have particular type of metabolic pathway to promote tumor growth which is called as Warburg effect. Most frequently exhibited feature of this effect is increased glucose uptake and fermentation of glucose to lactate. Tumor cells consume more glucose compared with normal cells, in recent years, many studies demonstrates that the 18F-fluro-D-glucose positron emission tomography/computed tomography (18F-FDG PET/CT used in tumor diagnosis, prognosis prediction and assessment of the therapeutic response through analysis of tumor metabolic activity [105-108]. Mitochondrial protein and mitochondrial DNA (mtDNA) are of huge significance for generating energy in both tumor cell and normal cell [109]. Scientifically proven, mtDNA sequence alteration may leads to tumor progression and metastasis [110]. This altered sequence of mtDNA has been noticed in multiple kinds of cancers [111]. This gives you an idea about analysis of mitochondrial constituents are significant in therapeutic target in cancer patients. For example, mitochondrial proteins such as Mitofusin- 2, Lon protease, and TFAM play key role in potential therapeutic target for bladder cancer [112]. In contrast with nuclear DNA, mtDNA has high mutation rate, higher copy number per cell which validates tumor specific circulating mtDNA a prospective biomarker of tumor "liquid profiling"[113]. Current research findings demonstrates that the analysis of circulating mtDNA content and mutants of tumor-specific mtDNA can be used as non invasive tool to identify the risk of developing bladder [114] cancer and hepatocellular cancers in HBV infection patients[115]. Research findings on prostate cancer patient's shows high levels of plasma mtDNA correlates with poor survival (less than 2years), which signify that circulating mtDNA can be used as marker for prognosis [116]. Mitochondrial single nucleotide polymorphism can be accurately analyzed with the help of exome sequencing [117], though its measurement in blood and current method of estimation remain uncertain.

Perspectives

In order to find out tumor specific markers in the circulation, ctDNA and circulating tumor cells are promising area of identifying cancer. One of the significant advantage of ctDNA analysis is altered genomic sequence present in it determines individual cancer and its absence in normal matched DNA. In terms sensitivity, high levels of circulating ctDNA are present in most advanced cancer patients, allows prediction of genetic determinants for treatment, monitoring tumor dynamics, and molecular heterogeneity, and finds roadway of genomic evolution and progress of acquired resistance. Cancer finding investigations like CTCs and ctDNA in circulation appears to be complementary with each other in cost effective and cancer associated hot spots. Analysis of CTCs in circulation is labor intensive and highly sensitive result giving tool to evaluate tumor heterogeneity, genome mutations in wide scale, drug sensitivity, and RNA and protein expression. Further, for early identification of cancer highly motivated efforts have been put forward to use liquid profile for improved disease outcome [118] and premature cancer onset. Significantly, current studies show that analytical sensitivity can be improved by isolation of RNA in exosomes along with ctDNA, instead of analyzing ctDNA alone [119]. Moreover, executing liquid profiling in clinical setting in large scale need comprehensive understanding on potential usage and its limitations, this detect various cancers in across multiple centers of large cohort patients.

Abbreviations

CfDNA: cell free DNA; ctDNA: circulating tumor DNA; CTC: circulating tumor cells; MiRNA: Micro RNA; FDA: Food drug administration; Ep-CAM: anti-epithelial adhesion molecule; CSF: Cerebrospinal fluid; CNVs: Copy number variations; EGFR: Epidermal growth factor receptor; ARMS: amplification refractory mutation system; PAPS: Pyro phosphorolysis-activated polymerization; TAM-seq: tagged-amplicon deep sequencing; BEAMing: Beads emulsion and amplification and magnetic's; PCR: polymerization chain reaction; EMT: epithelial-to-mesenchymal; FFPE: formalin-fixed paraffin-embedded; TNM: Tumor, Nodes, Metastasis;CTscan: computerized tomography scan; EGFR-TRI: epidermal growth factor receptor tyrosinase kinase inhibitor; BRCA: Breast cancer gene A; PARPi: poly (ADP)-ribose polymerase; PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit; CDK4: cyclin dependent kinase 4; PFS: progression-free survival; AR: androgen receptor; FGFR: Fibroblast growth factor receptor; ICC: intrahepatic cholangiocarcinoma; EpCAM: Epithelial cell adhesion molecule; mCRPC: Metastatic castration-resistant prostate cancer; NGS: next generation sequencing;mtDNA: Mitochondrial deoxy ribo nucleic acid; 18F-FDG PET/CT: 18F-fluro-D-glucose positron emission tomography/computed tomography;TFAM: Mitochondrial transcription factor A; HBV: Hepatitis B Virus.

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Competing interests

The author declares that they have no competing interest

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