

Review Article

ctDNA Detection in Microfluidic Platform: A Promising Biomarker for Personalized Cancer Chemotherapy

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Received 27 August 2019; Accepted 10 December 2019; Published 8 April 2020

Academic Editor: Luca De Stefano

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Early detection and characterization of circulating tumor DNA (ctDNA) can reveal mint of comprehensive biological insights from indicating the presence of tumor, identifying mutational changes of malignant cells, and allowing precision or targeted therapy together with monitoring disease progression, treatment resistance, and relapse of the disease. Apart from these, one of the greatest axiomatic implications of ctDNA detection is that it provides a new shed of light as noninvasive liquid biopsy as a replaceable procedure of surgical tumor biopsy. Despite the tremendous potential of ctDNA in cancer research, there remains a paucity of quantitative study on ctDNA detection and analysis. The majority of previously published microfluidic-based studies have focused on circulating tumor cell (CTC) detection and have failed to address the potential of ctDNA. The studies on microfluidic ctDNA detection are not consistent might be due to the complexity of ctDNA isolation as they present in low concentration in blood plasma. Researchers need to leverage the ability of microfluidic system for ctDNA analysis so that the significant enigma about cancer can be resolved effectively. This study, therefore, highlights the importance of ctDNA as cancer biomarker for liquid biopsy and provides an overview of the current laboratory as well as microfluidic techniques for ctDNA detection. This paper also attempts to show the emergence of new strands of microfluidic ctDNA detection and analysis for personalized cancer chemotherapy.

1. Introduction

A biomarker is a biomolecule found in body fluids or tissues that describes a normal or abnormal biological state of condition or disease. The World Health Organization [1] defined biomarker as biological substance, structure, or process that can be measured in the body for disease prediction [2]. Generally, the potential biomolecules as biomarkers are proteins (enzyme or receptor), peptides, nuclei acids (DNA, RNA), antibodies, and alterations such as gene expressions, proteomic signatures, metabolomic signatures, and cell mutations [3]. In cancer research, cancer biomarkers are biomolecules that secreted by tumor and responding specifically due to the presence of tumor. Recently, the most studied and effectively detectable cancer biomarkers are circulating tumor cells (CTCs) and circulating tumor DNAs (ctDNA). These biomarkers can be identified in the circulation of whole

blood, serum, and plasma or in other body fluid such as urine and saliva [3]. Apart from predicting disease as a prognostic biomarker, it also measures the risk of cancer progression, response towards treatments, and pharmacogenomic response (response of drug by cells). This term is also known as a diagnostic biomarker used in personalized medicine where precise treatment or therapy can be provided without adverse reaction [2, 4].

Circulating tumor cells (CTCs) are cells that originate and disgorge from primary tumor, recurrences, or metastases that are circulating freely in the bloodstream [5]. They have specific genetic characteristic of the primary tumor and metastasize to various part of the organ even in primary stage [6]. Based on several reviews, CTCs can be identified at the early stage of tumor growth and this can be therapeutic biomarker for treatment evaluation as well as detecting metastatic relapse of a particular disease or tumor [7, 8]. CTCs may

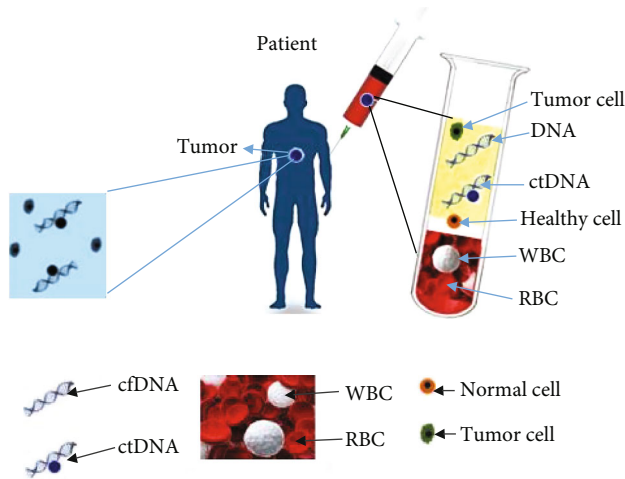


FIGURE 1: Components in blood sample of a cancer patient after the centrifugation process. White blood cells (WBC) and red blood cells (RBC) can be found in red blood, while normal cells, tumor cells, cfDNA, and ctDNA can be found in plasma.

release from various tumor of various part of the organ which varies in nature. Thus, CTCs can display heterogeneous cells that exuviate from primary tumor which an additional analysis is needed to study the characteristic and location of the primary tumor. Recently, CTCs isolated based on promising noninvasive liquid biopsy technique and have been validated as potential biomarkers in diversity of cancers including lung, colorectal, breast, and prostate [9]. However, the concentration of CTCs in the bloodstream is very low as such 1 in 10^9 of blood cells and only a particular number of CTCs able to generate metastases. Accordingly, there is a need for further analysis to characterize metastatic and nonmetastatic CTCs for appropriate precise therapy [5, 10].

Circulating cell-free DNA (cfDNA) is degraded small DNA fragments found circulating freely in the bloodstream (plasma and serum) and other body fluids. It is mostly double-stranded extracellular molecules of DNA with smaller fragments around 70 bp to 200 bp which do not necessarily originate from the tumor [11, 12]. Based on earlier studies, researchers found that the presence of cfDNA is significantly higher in cancer patients compared to healthy patients. The elevated number of cfDNA contains a diluted number of tumor-specific mutation which also known as circulating tumor DNA (ctDNA) [13]. ctDNA is a biomarker released from cancerous cells and tumors as they grow and replaced by new ones. The number of ctDNA difference depends on tumor type, location, and tumor cascade. The overall picture of CTCs, cfDNA, and ctDNA is shown in Figure 1.

Analysis of ctDNA provides revelation about heterogeneous information for detecting tumor by liquid biopsy, providing more effective precision therapy and monitoring disease condition including nonhematological as well as progression [14]. Cancer can be categorized as an incommensurable disease where the molecular mechanism of tumor eventually responses over tumor-stage progression, treatment, or immune system changes during therapy. Almost all chemotherapies have the resistance criteria and lead to tumor clonal evolution for the duration of disease progres-

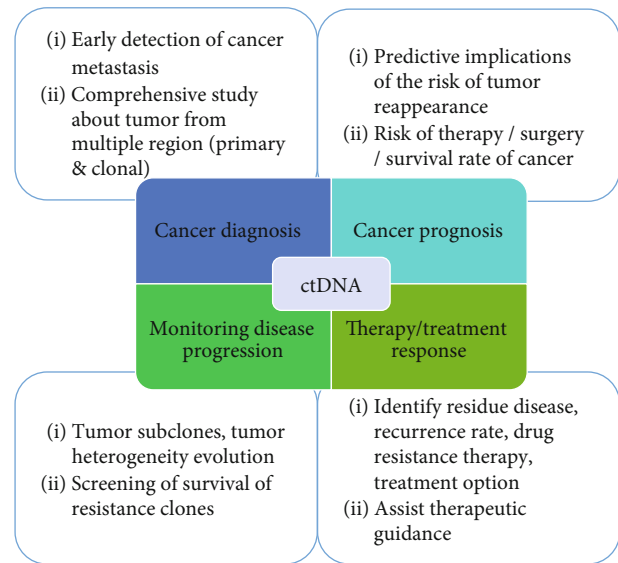


FIGURE 2: The potential clinical benefits of ctDNA detection as a promising biomarker for cancer treatment and management.

sion [15]. As ctDNA is released from multiple tumor regions including primary and clonal, the analysis provides voluminous information regarding tumor heterogeneity and clonal evolution. A great deal of previous research has recognized the feasibility of ctDNA in detection of tumor residual, survival of resistant clones, and drug resistance over treatment and predicts emerging subclones before recurrence of tumor [16–18]. Moreover, early detection of metastasis throughout tumor progression is possible by ctDNA. ctDNA alteration and the escalating number of ctDNA in early-stage also predict the probability of disease recurrence [19–21]. Some cross-sectional studies about ctDNA predicted the patient prognosis when treated with surgery, chemotherapy, or radiotherapy and risk of tumor reappearance [22, 23]. The study furthermore can be used to monitor treatment response rate for better cure management and therapeutic guidance [24, 25]. It has conclusively been shown that ctDNA detection is comparative to cancer stage. The detection rate is 100% for II-IV compared to stage I which is 50% for non-small-cell lung cancer (NSCLC) [18, 26]. The inclusive potential clinical benefits of ctDNA detection are described in Figure 2.

Data from several sources have stated that it is easier to separate ctDNA than CTCs as ctDNA is more stable and higher in proportion in the bloodstream with high sensitivity. Only few CTCs are detectable in a single blood draw of 7.5 ml, whereas cfDNA including ctDNA is also released by apoptotic cells and tumor cells [21, 27]. Their concentration is directly proportional to the tumor cascade [28]. In spite of that, further isolation procedure of methylation analysis is needed to well define tumor-specific cfDNA or particularly ctDNA. Methylation analysis is DNA chromosomal pattern study by the methyl group. The patterns of genome changes in methylated DNA show the exact tumor-specific DNA particularly ctDNA or biomarker identification [29, 30]. The identification of suitable cancer biomarker hinges the pathway towards tangible personalized medicine. The escalating personalized medicine in revolutionizing healthcare enables

to deliver the right treatment for the right person at the right time for disease diagnosis screening, monitoring tumor progression, and drug resistance reactions [31]. Analysis of ctDNA as cancer biomarker is amenable for noninvasive liquid biopsy which gives quick access to tumor sample rather than conventional tissue biopsy which needed surgical examination of tumor tissue. In spite of the fact that ctDNA highlighted many flourishing possibilities as well-validated prognostic and diagnostic biomarker in cancer research, yet the emergence of a high-sensitive and selective point-of-care device to leverage the potential of ctDNA is still needed. Microfluidic platform as a healthcare device plays an essential role in addressing the needs for modern molecular technology in manipulating various analyses for DNA, proteins, and cells simultaneously or consecutively depending on requirements. Hence, this review summarizes the current laboratory liquid biopsy techniques, developed microfluidic liquid biopsy devices, and future trends for rapid detection and characterization of ctDNA in cancer patients.

2. Methods of ctDNA Analysis

Identification and analysis of ctDNA typically have been carried out in multiple laboratories which required large equipment and professional skills to handle the sample and it is considered time-consuming procedures. Consequently, ctDNA rapid detection and analysis was developed from the laboratory (macroscale analysis using a large volume of sample) to microfluidic techniques (microscale analysis using a small volume of sample). The development of this analysis is described in the following part.

3. Laboratory Liquid Biopsy Techniques

A biopsy is an investigation of cell or tissue taken surgically from tumor to study and confirm the presence, cause, and stage of a tumor or disease. Conventional tissue biopsy is clinically not worth considering for some diseases screening mainly for lung and brain cancers. Surgically removing part of tissue may cause of nerve injury and bleeding or disease spreading. Moreover, surgery during chemotherapy where the genome of the tumor cell is highly unstable for continuous monitoring of medication and tumor progress is unbearable [32]. In contrast, liquid biopsy where analysis is based on fluid sample such as blood offers noninvasive continuous heterogeneous and comprehensive disease observation technique. Diversity of required information about the type, stage of the tumor, and chemotherapy progress from personalized medicine can be accessible from time to time through detection of ctDNA by this liquid biopsy [33]. The required procedures for tissue biopsy and liquid biopsy are described as a comparison in Figures 3 and 4.

Current laboratory technique and analysis of ctDNA can be divided into four categories. They are sequencing, polymerase chain reaction (PCR) including Scorpion Amplification Refractory Mutation System (ARMS), beads, emulsions, amplification and magnetic analysis (BEAMing), and next-generation sequencing (NGS) [32, 34, 35]. PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), or

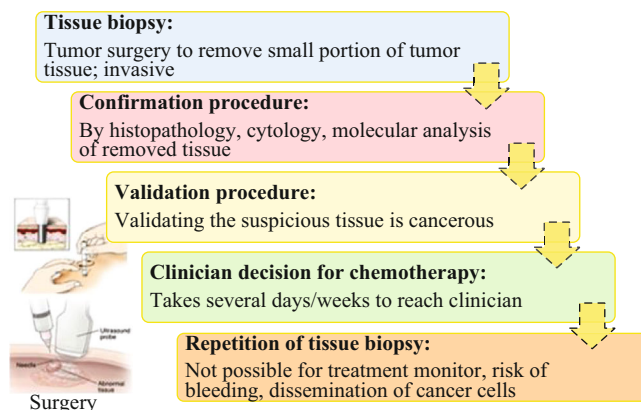


FIGURE 3: The overall procedures of tissue biopsy only provide a static tumoral snapshot of a specific time point and do not reflect dynamic changes that occur during cancer treatment.

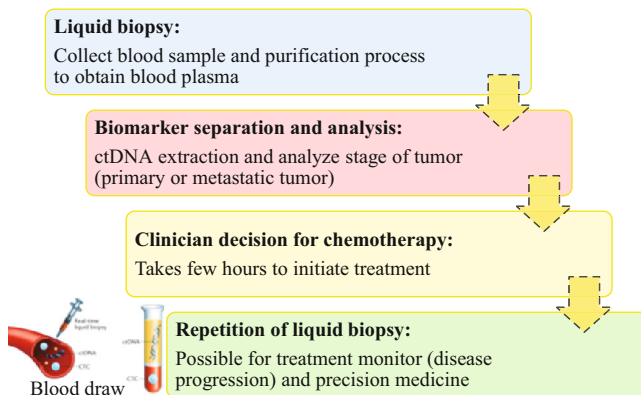


FIGURE 4: The overall process of liquid biopsy as noninvasive process that is possible to assess the dynamic tumoral changes by ctDNA analysis over the course of therapy and possibilities for early therapy before the tumor spreading.

improved version of droplet digital PCR (ddPCR) is modified from a conventional method to study genetic alteration, amplify, and detect rare known mutant DNA molecules as well as known allele-specific mutation [36]. PCR and qPCR have been the most widely used methods for quantification of nucleic acid. Nevertheless, they were considered as insensitive to detect low concentration of the mutant allele and the amplification initiates when only the required amount of targeted allele is present in the sample. Thus, researchers developed Scorpion ARMS integrated with real-time qPCR to detect low concentrated mutant allele [37, 38]. Then, ddPCR was introduced where it amplified targeted DNA templates only from the whole sample and the presence of analytes sequence was shown through the positive signal indicator. This method was considered as high insensitive by reducing false-negative rates which are typically observed by qPCR-based techniques [39]. Similarly, ddPCR and BEAMing are also based on emulsion and amplification where isolation of required template into thousands of tiny droplets of the reaction chamber for amplification [39]. BEAMing with the same principle as ddPCR binds DNA to magnetic microbeads before emulsion into droplets. The

method is based on sorting beads that contain cell mutation by using flow cytometry [40]. After the amplification process, magnetic microbeads are eluted out for purification of PCR product and mutation can be detected [41]. Although the detection capacity for various stages of cancer and sensitivity is quite high for Scorpion ARMS, ddPCR, and BEAMing, but these methods are only applicable for known mutations only and procedures are complex for clinical setting [42]. Numerous studies have presented the detection of ctDNA in cancer patient, but due to practical limitation in clinical application, sequencing and NGS methods were developed. These methods allowed ctDNA detection by parallel sequencing of hundreds of millions of DNA fragments from a single sample. The parallel sequencing is also used for simultaneous detection of varieties of potential mutations. NGS analysis also can be used to assist abundance of clinical decision-making from biomarker detection, analysis for diagnosis, prognosis, and monitoring of cancer treatments [43–46].

In an analytical study based on the mentioned current laboratory techniques, the percentage of detectable ctDNA in tumors varies according to stages as 49% to 78% of sensitivity in localized tumors while 86% to 100% of sensitivity in metastatic tumors [47]. Ishii and his group [48] detected drug resistance mechanism in epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) treatment for non-small-cell lung cancer (NSCLC). The analysis with 81.8% sensitivity plus 85.7% specificity was based on collected plasma (ctDNA) and tumor sample and conducted using droplet digital PCR (ddPCR). EGRF mutant in non-small-cell lung cancer (NSCLC) with analysis of 81% sensitivity and 85% specificity was detected by Gray et al. [24]. Another few studies presented ctDNA detection and confirmed the presence of colorectal cancer [49] and ctDNA of breast cancer [50]. Ashida et al. [51] successfully detected rare mutant DNA molecules and specified allele by PCR amplification. van Ginkel and his colleagues [52] identified ctDNA and specified rare mutational marker by ddPCR and BEAMing using a blood sample. Sequencing either whole genome sequencing (WGS) or whole exome sequencing (WES) is a comprehensive study of the tumor genome for rare mutation identification, therefore, assisting in clinical treatments [35, 53]. Although WGS is used to identify malignant mutation, the changes in the primary tumor due to given therapy affect the subsequent analysis of the tumor genome. Similarly, the high-yield WES is used to detect rare disease and tumor cell mutation but is only applicable at later stage of tumor detection. The next-generation sequencing presented numerous advantages from reduced sequencing time, cost, and detail analysis of genome by simultaneous sequencing of millions of DNA molecules. The first modified method of sequencing is known as tagged-amplicon deep sequencing (TAm-Seq) which was used to identify the presence of metastatic mutations in patients with advanced ovarian cancer. Then, the capture-based deep sequencing method was introduced by Newman et al. [54] and developed together with Bratman et al. [26] to identify ctDNA in multiple mutations with comprehensive diagnostic information, used to monitor residual disease and detected single nucleotide variants. Then, this method was further improvised by

Newman and his group in [55] to digital error suppression-enhanced deep sequencing mainly to increase the sensitivity and specificity of ctDNA detection. Volik et al. [13] demonstrated Safe-Sequencing System (Safe-SeqS), based on amplicon approach for effective detection of rare variants and able to identify ctDNA in many tumor types by reducing the sequencing error rate. However, this required a sophisticated data analysis system with patient-specific optimization of amplicon for alteration such as somatic copy number and fragment rearrangement detection. In a follow-up study, WGS was used to analyse ctDNA in patients with metastatic breast cancer and treatment options were studied too. By the same token, WES was used for ctDNA detection in patients with advanced breast, ovarian, and lung cancer [56]. The summary of the laboratory method for ctDNA detection is shown in Table 1. Although these methods are sensitive in ctDNA detection and monitor disease progression, but they remain expensive and time-intensive and require sophisticated data analysis systems. Thus, clinical microfluidic device has shed a new light for rapid, cost-effective, and most importantly with simple workflow where this technique could be repeated anytime anywhere for molecular analysis as shown in Figure 5.

4. Microfluidic Liquid Biopsy Techniques

The past decade has seen the rapid development of microfluidic system for ranges of healthcare considerations. A single microfluidic chip is able to perform series of analysis or experiments conducted in an entire lab which requires several days to reveal the output. As the required sample and reagent volume is small, the microfluidic platform might be applicable for analysis of a single cell. Mostly, microfluidic device can be created as a portable, transportable, pocket-sized tool and also diminutive bench top instrument. It would be a great supporting device to deliver the right care at the right time. However, there are some considerations in the development of the microfluidic devices for medical essential. The poor quality of analysis and result interpretation will directly lead to an inaccurate treatment option thus may affect patient's health. Although the raw specimen input to the device does not necessitate the centrifugation process, yet the quality of specimen and analytical technique play an imperative role to produce high-yield result.

There is a relatively small body of academic literature concerned with ctDNA detection via microfluidic. This is essentially due to insensitive detection of low concentrated ctDNA in the background of nonmutated DNA from normal cells. Major advances in microfluidic have revealed the emergence of application in molecular and biomedical for rapid analysis of clinical usage. Size and properties of ctDNA are one of the ideas to initiate the development of microfluidic ctDNA detection. Accordingly, electrokinetic trapping on microchannel was introduced which relies on the accumulation of charged ions by applied electric field forming the depletion of targeted analytes [57]. In a follow-up study, size-based micropillar structure on microchannel, microcolumn-packed separation, and microchannel with the electrophoretic system [58, 59] were developed for ctDNA extraction to achieve high yield

TABLE 1: Laboratory methods for ctDNA detection to identify rare mutational marker and the presence of tumor.

Method	Description	Limitations	References
Whole genome sequencing	Deep sequencing of the genome	Expensive, limited to SCNA detection with low insensitivity	[53]
Whole exome sequencing	Deep sequencing of the exome	Expensive and low insensitivity	[53]
Next-generation sequencing	Capture-based deep sequencing for detecting 95% of tumor in NSCLC. Detection specificity of 96% for mutant allele fraction in 100% of stages II-IV patients and 50% of stage I patients	Less comprehensive than whole genome/exome sequencing	[26, 54]
	Detect EGRF mutant in NSCLC with analysis of 81% sensitivity and 85% specificity		[24]
	Detect ctDNA and confirm the presence melanoma skin cancer		[48]
Digital or droplet digital PRC	Detect ctDNA and confirm the presence of colorectal cancer	Detection limit to a small number of genomic positions in the sample	[49]
	Detect ctDNA and confirm the presence of breast cancer		[50]
	Detect rare mutation marker		[52]
PCR	Amplifying rare mutant DNA molecules and detecting allele-specific mutation	Lower sensitivity, detection limit to a small number of genomic positions in the sample	[51]
Modified next-generation sequencing	Digital error suppression-enhanced deep sequencing for 90% of sensitivity, 96% specificity in mutation EGFR kinase domain detection	Less comprehensive than whole genome/exome sequencing	[55]
BEAMing	Evaluate specifically RAS mutation in the blood	Evaluate only known cell mutations	[42]

Note: SCNA: somatic copy number alteration; NSCLC: non-small-cell lung cancer; EGRF: epidermal growth factor receptor; PCR: polymerase chain reaction; BEAMing: beads, emulsions, amplification, and magnetics.

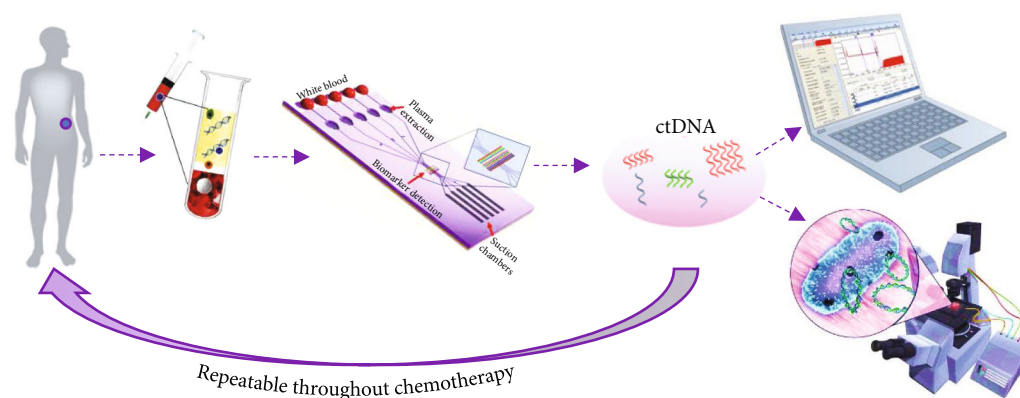


FIGURE 5: Microfluidic point of view for noninvasive liquid biopsy of ctDNA detection and analysis.

and purification. These methods have potential to overcome most of the microfluidic challenges such as improves heat dissipation, reduces cfDNA separation duration by application of large electric field, and reduces the amount of required blood sample from patient and related buffer solution for separation. One of the major limitations encountered by these methods is the separated cfDNA that could not further be purified for specific ctDNA detection. Followed by this method, concentrated ctDNA-based microfluidic particularly a narrow microchannel [60] and nanopore microchannel [61] were modified for the enrichment of ctDNA separation. Besides, another earlier development of microfluidic studies for cfDNA or ctDNA

detection was based on conventional qPCR technique applied in microscale platform. Thermal amplification of a single DNA copy and real-time fluorescence was used to monitor DNA concentration with the help of intercalating dyes, e.g., SYBR Green, or DNA fluorescent probes, e.g., TaqMan. Microfluidic system with thermal amplification as qPCR and droplet-based reaction chamber which represents ddPCR were reported for cfDNA detection without sensitivity specification of the fabricated devices [62, 63]. Chaudhuri et al. reported seven droplet-based digital PCR microfluidic system to identify somatic mutations by detection of ctDNA [64]. The droplet-based microfluidic with TaqMan® probes

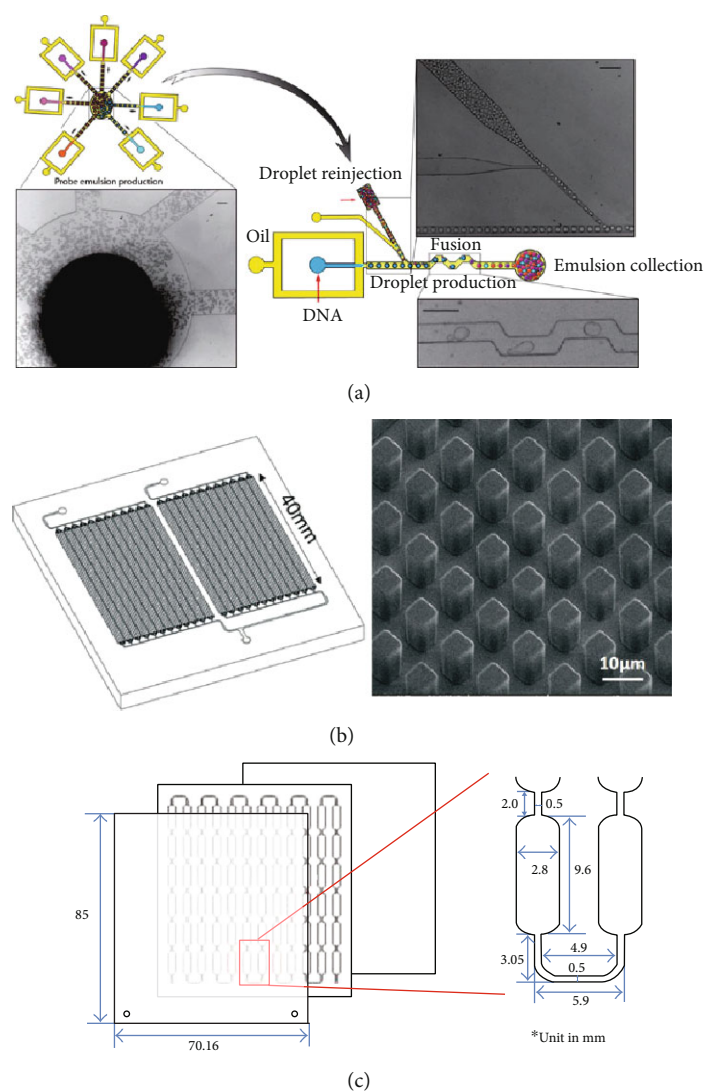


FIGURE 6: (a) Digital PCR-based microfluidic device with seven droplets to detect wild-type mutation of the *KRAS* oncogene [64]. (b) Micropillar-based plastic microfluidic surface to detect cfDNA; more than 90% of purities of cfDNA was extracted [69]. (c) Microcolumn- and microwell-based microfluidic for cfDNA isolation [71].

was used to isolate mutant DNA from wild-type DNA with fluorescent signals. Accurate and sensitive quantification of mutated *KRAS* oncogene was detected, although this platform is limited by the number of droplets for analysis. Bahga et al. [65] developed electrodes with dielectrophoretic capture trapping on microfluidic to separate ctDNA, and high-yields results were reported without particular details of sensitivity level of the device [66]. Microfluidic fluorescence in situ hybridization (FISH) assay was fabricated based on conventional FISH technology to screen hematopoietic malignancies. The device detected minimal residual disease can analyze 10 samples in submicroliter volume at a time [67].

To the extent of our knowledge, previous research findings on ctDNA detection by microfluidic have been limited, inconsistent, and contradictory from the past decade up to 2017. As a breakthrough, in 2017, Koboldt et al. proposed microfluidic multiplex PCR sequencing technology for high-throughput and sensitive quantitation of ctDNA

[68]. A multiplex PCR preamplification integrated with sequencing method for quantifying low concentrated ctDNA and continued by off-chip empirical Bayesian model to study error specific to characterize ctDNA; thus, precision approach can be taken to revise their work. They reported ctDNA mutation detection and disease monitoring using plasma of ovarian and pancreatic cancer patients (clinical trial samples) with sensitivity of 92% and specificity of 100% which matched with tumor tissue. A novel low-cost plastic microfluidic surface based on solid phase microextraction (SP μ E) for cfDNA extraction was demonstrated by Campos et al. [69]. Typically, clinical analytes of interest can be extracted by solid phase extraction in microfluidic by several methods using micropillar structure, porous solid phase, or immobilized magnetic beads [70]. Campos et al. (2018) used array of micropillars to increase extraction bed load (scalable to loads >700 ng of cfDNA) and immobilization buffer (IB) consisting of polyethylene glycol and salts that induce cfDNA condensation onto the

TABLE 2: Microfluidic methods for ctDNA detection to identify rare mutational marker and presence tumor.

Method	Description	Limitations/achievements	References
Electrokinetic trapping	Separation based on properties of cfDNA, depletion of cfDNA based on charged ions	cfDNA could not further purified for specific ctDNA detection.	[57]
Micropillar, microcolumn packed, electrophoretic system on microchannel	Separation based on size of cfDNA	Insensitivity of the microdevice; cfDNA could not further be purified for specific ctDNA detection.	[58, 59]
ctDNA concentration-based microfluidic channel	Narrow microchannel and nanopore microchannel for ctDNA separation	No sensitivity specification	[60, 61]
qPCR and droplet-based reaction chamber	Thermal amplification which represents ddPCR was reported for cfDNA	No sensitivity specification	[62, 63]
Seven droplet-based digital PCR microfluidic system	Identify somatic mutations by detection of ctDNA. Sensitive quantification of mutated <i>KRAS</i> oncogene	Limited by the number of droplets for analysis	[64]
Dielectrophoretic capture	Electrodes used for trapping on microfluidic to separate ctDNA	No sensitivity specification	[65]
Microfluidic multiplex PCR	Integrated with sequencing technology for ctDNA mutation detection and disease monitoring using plasma of ovarian and pancreatic cancer patients	Sensitivity of 92% and specificity of 100%	[68]
Plastic microfluidic surface	Micropillars and immobilization buffer (IB) used for cfDNA isolation from samples of colorectal and non-small-cell lung cancer patients to detect <i>KRAS</i> mutation gene	90% of purities in cfDNA	[69]
Microfluidic platform integrated with Sanger sequencing method	Dimethyl dithiobispropionimidate (DTBP) binds to the amine group of ctDNA and sodium bicarbonate was used as an elution buffer to isolate ctDNA.	Identified 71.4% of mutation profile of <i>KRAS</i> and <i>BRAF</i> from colorectal cancer of patients stages I to IV within 15 minutes	[71]

activated plastic microfluidic surface. More than 90% of purities in cfDNA extraction was noticed and the use of IB reduced the interference of coextracted genomic DNA in the final output. The chip also proved can be utilized for clinical disease detection by extracted cfDNA from plasma samples of colorectal and non-small-cell lung cancer patients to detect *KRAS* mutation gene. Another new method was introduced by Aravanis et al. by sampling cfDNA from blood plasma of colorectal cancer patients for ctDNA detection [71]. The microwells and microcolumn-based microfluidic platform used dimethyl dithiobispropionimidate (DTBP) for ctDNA isolation and integrated with Sanger sequencing method for ctDNA validation. The outcome from this research showed that DTBP tremendously reduces detection of cellular background particularly DNA that might be released from noncancerous cell during chemical mixing or elution process. DTBP with amine-reactive homobifunctional imidoester (HI) binds to the amine group of ctDNA and sodium bicarbonate was used as an elution buffer to isolate ctDNA. This microfluidic platform identified 71.4% of mutation profile of *KRAS* and *BRAF* from colorectal cancer of patient stages I to IV within 15 minutes. Few examples of microfluidic system for isolation and detection ctDNA are shown in Figure 6.

The summary of microfluidic method for ctDNA detection is shown in Table 2.

5. Conclusions

The identification of suitable cancer biomarker hinges the pathway towards tangible personalized medicine. Analysis of ctDNA as cancer biomarker is amenable for noninvasive liquid biopsy which gives quick access to tumor sample rather than conventional tissue biopsy which needed surgical examination of tumor tissue. In spite of the fact that ctDNA highlighted many flourishing possibilities as well-validated prognostic and diagnostic biomarker in cancer research, yet the emergence of high sensitive and selective microfluidic device in healthcare to leverage the potential of ctDNA is still needed. As there is very limited and inconsistent research on ctDNA detection via microfluidic, a more cost-effective and time-saving microfluidic platform is necessary to cope with this era of precision medicine. In a nutshell, microfluidic analysis of ctDNA would give quick access to tumor sample rather than conventional tissue biopsy helpful in clinical setting for rapid and early ctDNA detection and appropriate treatment can be prescribed.

6. Future Perspectives

Looking forward, the current challenges of microfluidic platform for ctDNA analysis are quite thrilling. Microfluidic platform for multiplex identification of biomarker has the forthcoming in quick disease detection and diagnostic. Simultaneous detection and analysis of multibiomarker would be an eye-catching approach in microfluidic research as they contain the fingerprint information of tumor or any other related disease and this feasibly improves the diagnosis accuracy. Besides, terahertz (THz) metamaterials have unique characteristics for biosensing application which are engineered to have properties that are impossible to obtain in natural materials. However, the structures of metamaterials are enormously complicated to fabricate. THz metamaterial biosensor works based on tuneable resonance vibrational frequency of biomarkers and electromagnetic (EM) wave. The metamaterial is structured with optimized permittivity ϵ , conductivity σ , and dielectric material property. One of the main abilities of this biosensor is in the identification of targeted biomarker which caused hypersensitivity reaction due to the consumption of drug and antibiotic. The fluctuations in water content of biological cells and tissues display changes in the permittivity ϵ and conductivity σ values of cells and tissues. The variation of permittivity ϵ and conductivity σ values of cells in the metamaterial leads to a high-sensitive resonant frequency which can be used to detect biomarkers. Integrating THz metamaterial into bioanalytical device could improve the detection sensitivity and limits to single molecules.

Abbreviations

DNA:	Deoxyribonucleic acid
CTC:	Circulating tumor cell
ctDNA:	Circulating tumor DNA
RNA:	Ribonucleic acid
cfDNA:	Circulating cell-free DNA
NSCLC:	Non-small-cell lung cancer
PCR:	Polymerase chain reaction
ARMS:	Scorpion amplification refractory mutation system
qPCR:	Quantitative polymerase chain reaction
NGS:	Next-generation sequencing
BEAMing:	Beads, emulsions, amplification, and magnetic
DPCR:	Digital polymerase chain reaction
ddPCR:	Droplet digital polymerase chain reaction
EGFR:	Epidermal growth factor receptor
TKI:	Tyrosine kinase inhibitors
WGS:	Whole genome sequencing
WES:	Whole exome sequencing
TAm-Seq:	Tagged-amplicon deep sequencing
Safe-Seq:	Safe-sequencing system
FISH:	Fluorescence in situ hybridization
SP μ E:	Solid phase microextraction
IB:	Immobilization buffer
DTBP:	Dimethyl dithiobispropionimidate
HI:	Homobifunctional imidoester
THz:	Terahertz.

Conflicts of Interest

Authors declare no conflict of interest.

Acknowledgments

The research was supported by the Ministry of Higher Education of Malaysia and Universiti Teknologi Malaysia (grant nos. Q.J130000.21A2.04E82 and Q.J130000.2523.16H38). We thank them for funding this project and for their endless support.

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