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Review

# Rethinking liquid biopsy: Microfluidic assays for mobile tumor cells in human body fluids

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ABSTRACT

Traditionally, liquid biopsy is a blood test involving the harvesting of tumor materials from peripheral blood. Tumor cells from non-blood body fluids have always been clinically available in cytological examinations but limited for use in differential diagnosis due to the low sensitivity of conventional cytopathology. With the recent significant progress in microfluidic and downstream molecular technologies, liquid biopsies have now evolved to include harvesting tumor cells and DNA fragments in all kinds of *non-blood* body fluids. This expansion into general body fluids presages the notion that liquid biopsy could soon be used in competition, as well as, in complementarity with tissue biopsy. Preliminary research of fluid-harvested tumor materials to spot early-stage tumors, monitor disease progression for metastasis and recurrence, and detect chemoresistance have been reported. To reflect the propagation of tumor cells in non-blood body fluids, we introduced the term *Mobile Tumor Cells* (MTCs), in lieu of the widely accepted term of *circulating* tumor cells (CTCs) resident in the bloodstream. Our review starts with a discussion on the clinical significance of MTCs, followed by a presentation of microfluidic techniques for MTC capture and various strategies for their identification. Hopefully, the phenotypic and genomic data acquired from harvested MTCs can be used to guide and improve cancer treatment decisions.



## 1. Introduction

Liquid biopsy in the traditional sense, is a blood test that involves the sampling and analysis of biological materials harvested from the peripheral blood. In cancer research, these biomaterials could be circulating tumor cells and circulating tumor DNA; in cardiology, they include circulating endothelial cells and in prenatal studies, they are the cell-free fetal DNA. With the recent expansion of the technology to encompass all kinds of *non-blood* body fluids (ascites, pleural effusion, urine, cerebrospinal fluid, etc. (Fig. 1)), liquid biopsy is rapidly gaining traction for minimally invasive blood and non-blood tests for cancer research. With the rapid advancement in materials, microfluidic technologies for cell capture, advanced biomarkers for cell identification, high-fidelity molecular techniques for single cell sequencing, and the development of AI-based big-data analytics for bioinformatics, it will not be too long a wait for liquid biopsy to be adopted as a mainstream tool for early cancer diagnosis, personalized treatment, therapy monitoring and recurrence detection. Last June 2016, the Food and Drug Administration approved the first liquid biopsy test for use in cancer diagnosis [1]. Therefore, it would be timely and useful to rethink the role of liquid biopsy by providing a critical review of the technology to medical researchers and practitioners alike. Unlike tissue biopsy, liquid biopsy is regarded as minimally invasive, less risky and significantly cheaper; hence, it can be sampled a lot more frequently to achieve a better diagnostic and monitoring accuracy for a more effective treatment. Numerous review papers on liquid biopsy have been published but most are focused on the harvesting and processing of tumor materials sourced from the peripheral blood of cancer patients (e.g. circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA). Therefore, we restricted our

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**Abbreviations and acronyms**

| | |
|---|---|
| AKT | The encoded proteins of AKT are serine-threonine kinases in the protein kinase B (PKB) family |
| AMACR | alpha-methylacyl CoA racemase |
| ATCs | Ascites tumor cells |
| CD45 | Cluster of differentiation 45 or Protein tyrosine phosphatase, receptor type, C |
| CKs | Cytokeratins |
| CTCs | Circulating tumor cells |
| CTC-DNA | Circulating tumor cell DNA |
| ctDNA | Circulating tumor DNA |
| DAPI | 4′,6-Diamidino-2-phenylindole |
| DC | Deformability cytometry |
| DFF | Dean flow fractionation |
| DLD | Deterministic lateral displacement |
| DNA | Deoxyribonucleic acid |
| EGFR | Epidermal growth factor receptor |
| EMT | Epithelial-mesenchymal transition |
| EpCAM | Epithelial cell adhesion molecule |
| EphA2 | Ephrin type-A receptor 2 |
| FAS | FAS (TNFRSF6) is a death receptor on the surface of cells that leads to apoptosis (programmed cell death). The FAS gene is on chromosome 10q24.1 |
| FGFR3 | Fibroblast growth factor receptor 3 or CD333 |
| FISH | Fluorescence *in situ* hybridization |
| GNPs | Graphene nanoplates |
| GO | Graphene oxide |
| HER2 | Human epidermal growth factor receptor 2 |
| IF | Immunofluorescent |
| JAK2 | Janus kinase 2 |
| MPE | Malignant pleural effusion |
| MTCs | Mobile tumor cells |
| MUC1 | MUC1 gene encodes a mucin glycoprotein expressed in most epithelial cells |
| 2-NBDG | 2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose, a fluorescent tracer |
| NK | Natural killer |
| NSCLC | Non-small cell lung cancer |
| OCT4A | mRNA isoform of OCT4 or Octamer-binding transcription factor 4 (POU5F1) |
| PCR | Polymerase chain reaction |
| PFF | Pinched flow fractionation |
| PIK3CA | Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (P110α protein) |
| PSMA | prostate specific membrane antigen |
| RAS | RAS proteins are small GTPases that regulate cell growth, adhesion, proliferation, survival and differentiation. The 3 RAS isoforms, HRAS, NRAS and KRAS are the most common oncogenes in human cancers |
| RBCs | Red blood cells |
| SiNPs | Silicon nanopillars |
| STAT3 | Signal transducer and activator of transcription 3 |
| TRAP | Telomeric-repeat amplification protocol assay |
| TERT | Telomerase reverse transcriptase |
| TKI | Tyrosine kinase inhibitor |
| VDC | Vortex-mediated deformability cytometry |
| WBCs | White blood cells |

coverage to fluidic tumor materials involving CTCs but not the tumor-derived exosomes and oncoviruses (human papilloma viruses, Epstein-Barr virus, hepatitis B and C viruses, etc.). Further, most current techniques for the identification of the captured tumor cells are based on an immunofluorescent (IF) staining technique using appropriate biomarkers. For better accuracies, the surface staining approach can be supplemented by a downstream molecular analysis, which requires the capture of cells with intact cellular morphology. Hence, in this review, we considered DNA sourced from single tumor cells from either blood (CTC-DNA) or a non-blood body fluid (MTC-DNA). This whole-cell DNA is thought to be more refined than mutant DNA fragments (ctDNA) released into the bloodstream or a body fluid by dead tumor cells as the latter is often adulterated with DNA fragments from other dead cells. Nonetheless, ctDNA is a well-defined biomarker for cancer research and therefore, both CTC/MTC-DNA and ctDNA will continue to find acceptance by the research community.

With <1% of cancer cells released into blood circulation that eventually lead to metastases [2,3], it is clear that cancer metastasis via non-blood body fluids is more important than the hematogenous route for many types of cancer. Therefore, we would like to expand the traditional review of liquid biopsy to include non-blood body fluids. Also, since tumor cells in non-blood body fluids are not necessarily *circulating*, we would like to introduce a new term, which would more accurately reflects their ease of propagation in body fluids: *Mobile Tumor Cells* – they are indeed, highly mobile in a fluidic environment; in order to freely and easily spread within a host body. Our review starts with a discussion on the clinical significance of MTCs in non-blood body fluids, followed by a presentation of microfluidic techniques for MTC capture and strategies for their identification, and concludes with the hope that the phenotypic and genomic information acquired from harvested MTCs can be employed to guide and improve cancer treatment decisions. Since many of the approaches are adapted from established methods for CTC capture, we will also provide short references on microfluidic technologies for CTC capture whenever appropriate and a discussion of their differences.

## 2. Clinical significance of mobile tumor cells

Currently, the data from captured MTCs is usually limited to phenotypic information. When a malignancy is suspected, a cytological specimen of a non-blood body fluid is generally used to examine for the presence and enumeration of MTCs. In this section, we show that more information, including genetic mutations, can be elicited from MTCs to help predict treatment outcomes and guide cancer therapy. We will focus on three types of popular non-blood body fluids: the malignant ascites, malignant pleural effusion and urine.

### 2.1. Malignant ascites

Malignant ascites refers to excess accumulation of fluid in the peritoneal cavity accompanied with presence of tumor cells [4,5]. Tumor cells in malignant ascites are particularly important in the case of epithelial ovarian cancer whereby the disease primarily metastasizes to the peritoneal cavity and hematogenous dissemination rarely occurs [6]. Studying the biology of MTCs in ascites is crucial. It has been suggested that MTCs in ascites are resilient and nutrient-efficient on the basis that these cells are far away from