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Advancements in liquid biopsy and radiology techniques for early cancer detection: A review of current methods and future directions

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***Abstract*---**Aim: This review explores advancements in liquid biopsy techniques and radiological imaging for early cancer detection, highlighting their potential to improve diagnosis and treatment monitoring. Methods: We analyzed various liquid biopsy technologies, focusing on circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomes, along with radiological imaging techniques such as PET scans and MRI. Key methodologies include size-based enrichment, immunoaffinity-based isolation, microfluidic technologies, and advanced imaging modalities, as well as their clinical applications and limitations. Results: Liquid biopsies offer non-invasive alternatives to traditional tissue biopsies, facilitating real-time monitoring of tumor dynamics. Techniques such as droplet digital PCR and advanced microfluidic devices have enhanced the sensitivity and specificity of biomarker detection. Radiological imaging provides complementary insights into tumor location, size, and metabolic activity, enhancing the overall diagnostic accuracy. While methods like CellSearch® for CTC counting are FDA-approved, ongoing research aims to refine isolation techniques, validate biomarkers' clinical relevance, and integrate imaging findings through large-scale trials. Conclusion: The integration of liquid biopsies and radiological imaging into routine clinical practice holds promise for personalized cancer management, enabling early detection and improved treatment responses. Continued development of these technologies is crucial for enhancing patient outcomes.

***Keywords*---**liquid biopsy, cancer detection, circulating tumor cells, circulating tumor DNA, exosomes, radiological imaging, personalized medicine.

Introduction

Increasing the efficacy of treatment interventions while facilitating the early diagnosis of pathological disorders is the main goal of many pharmaceutical corporations and biomedical research endeavors. Tumor biopsy is the required method of obtaining tissue for cancer diagnosis and genetic assessments in clinical settings (1). This procedure is the invasive removal of malignant cells or tissue for examination. Tumor tissue is still the gold standard for identifying and diagnosing cancer, but obtaining and using it is still fraught with difficulties (2).

Sample bias can affect tissue-based tumor profiles, which can lead to an inaccurate representation of a tumor's heterogeneity and a limited understanding of its temporal and geographic aspects. Tissue biopsies are also not the best option for repeated sampling and may result in higher healthcare expenses. As an alternate technique for tumor genotyping, however, genomic profiling of materials generated from circulating cancer, sometimes known as "liquid biopsies," has become popular. The goal of liquid biopsies is to find tumor-specific genetic alterations by examining circulating biomarkers (CBs) extracted from body fluids such as blood or urine. Since CBs play a major part in many different cellular regulating processes, they are proven to be useful, non-invasive tools for monitoring the course of cancer treatment and prognosis. As such, the identification of relevant CBs indicating tumor behavior using non-invasive techniques represents a major advancement in the direction of individualized clinical management.

Liquid biopsy techniques have shown great promise in evaluating cancer patients' genetic profiles, monitoring treatment responses, and detecting the start of therapeutic resistance, as demonstrated by a number of studies (3, 4). Because liquid biopsies may be performed on the same patients repeatedly over time and sample collection methods are minimally invasive, molecular profiling of liquid biopsies for cancer biomarker identification is an intriguing research avenue. The growing need and understanding of genetic changes and tailored treatment options have led to a boom in the demand for molecular profiling techniques in recent years. It is crucial to use circulating tumor indicators in clinical settings and integrate them into routine clinical procedures. Therefore, in order to offer the solid data required for the clinical adoption of CBs, it is imperative that large-scale, prospective multicenter trials demonstrate the analytical specificity and clinical relevance of CBs. Through continued monitoring, this approach will also help discover variations that define cancer and minimize false positive outcomes (5).

Many CB analysis detection techniques have been developed recently for both clinical and research uses (6, 7). The main components of these techniques are the integration of nanomaterials, electrochemical systems intended for point-of-care use, and sophisticated miniaturized platforms with liquid biopsies. PCR and next-generation sequencing are the methods used in current ctDNA analysis technologies (NGS). More sensitive methods for ctDNA analysis have been developed as a result of the low ctDNA content in total cfDNA. These methods include droplet digital PCR (ddPCR) (8), digital PCR (dPCR) (9), and beads, emulsion, amplification, magnetics (BEAMing) (10). Only a few number of circulating tumor cell (CTC) technologies have been developed for clinical application, mostly due to difficulties with CTC heterogeneity and blood isolation (11). As of right now, the only FDA-approved clinical application for counting epithelial CTCs is the CellSearch® platform (12). For the purpose of examining exosomes and their contents, a number of detection techniques have also been developed, such as flow cytometry (14), enzyme-linked immunosorbent assay (ELISA) (13), and nanoparticle tracking analysis (NTA) (15). The usefulness of CTCs, ctDNA, and exosomes as cancer biomarkers (16–18) and the methods for detecting them have recently been covered in a number of reviews (5, 19, 20). This paper focuses on cutting-edge technologies and novel ideas, as well as the current

difficulties in implementing liquid biopsies in clinical practice. It also emphasizes the potential and clinical significance of CTCs, ctDNA, and exosomes as essential components of liquid biopsies.

In conclusion, liquid biopsies represent a transformative approach in cancer diagnosis and monitoring, addressing many limitations associated with traditional tissue biopsies. By leveraging non-invasive techniques to analyze circulating biomarkers, liquid biopsies offer valuable insights into tumor genetics, enabling real-time monitoring of treatment responses and the early detection of therapy resistance. As the demand for personalized medicine grows, the integration of circulating tumor biomarkers into routine clinical practice becomes increasingly vital. Continued research is essential to validate the analytical specificity and clinical utility of these biomarkers through large-scale studies. This will enhance our understanding of cancer dynamics and improve patient outcomes. Ultimately, the advancement of liquid biopsy technologies holds great promise for revolutionizing cancer care, making it more effective, less invasive, and tailored to individual patient needs.

Common Circulating Biomarkers' Biogenesis

Many prospective biomarkers with substantial therapeutic promise have been found as a result of recent advances in genomes, proteomics, and molecular pathology. Their use in the staging of cancer and in the provision of individualized care during diagnosis may improve patient outcomes. The ability to assess different biomarkers for early cancer detection and perform genomic and immune profiling is provided by liquid biopsies, which mainly involve circulating tumor nucleic acids (ctDNA and ctRNA), circulating tumor cells (CTCs), tumor-derived extracellular vesicles (tdEVs), autoantibodies, and tumor-educated platelets (TEPs). This makes it easier to choose the best course of treatment and track its effectiveness. Since Gold and Freedman discovered the cancer embryonic antigen (CEA) in colon tissue extracts in the 1970s, researchers have looked into cancer antigens as non-invasive indicators for early cancer detection (21, 22). Subsequently, additional cancer antigens that are clinically significant were discovered, including CA125 and PSA (23, 24). In the reviews that follow, the function of protein-based biomarkers in cancer detection is covered in great detail (25, 26). Particularly covered in this review are exosomes, ctDNA, and CTCs.

CTCs are thought of as tumor proxies that can monitor the development of metastatic disease and the response to treatment because the spread through blood and lymph is an important stage in the metastatic process (27). Australian physician Thomas Ashworth described CTCs for the first time in 1869 by contrasting their appearance with that of different tumor cells seen in cancer patients' blood (28). It was determined that tumor cells had the ability to infiltrate blood arteries and reach the circulation. Certain genetic indicators, such as mutations, chromosomal abnormalities, and gene expression profiles, are associated with the spread of cancer (29–31). As a result, CTC analysis concentrates on comprehending the mechanisms underlying cancer spread. The multi-step process known as the invasion-metastasis cascade, which comprises local invasion, intravasation into circulation, and the development of micrometastatic colonies, is responsible for the dissemination of cancer cells from

primary tumors and their seeding in distant regions (32). The epithelial-to-mesenchymal transition (EMT), a reversible alteration marked by decreased cell adherence and enhanced motility and invasiveness, is a central notion concerning intravasation processes. Interleukin-6 (IL-6), WNT, TGF-beta, platelet-derived growth factors, and extrinsic stimuli including alcohol, nicotine, and UV light can all cause EMT (33–35).

Exosomes

1946 saw the publication of the first noteworthy study demonstrating the existence of membrane-enclosed vesicles (36). Originally thought to be "platelet dust" or cellular debris expelled from the plasma membrane (37), studies conducted in the 1980s discovered a more intricate secretion mechanism involving tiny extracellular vesicles with a diameter of roughly 50 nm (38, 39). These extracellular vesicles produced from endosomes were first referred to as "exosomes" in 1987 (40). Until 1996, when it was shown that B-immune cell-secreted exosomes could specifically activate CD4+ T-cell clones against certain antigens, their precise roles were unknown (41, 42). Since then, different extracellular vesicles have been classified according to their biogenesis, functions, and cellular origin (43, 44). Several processes are involved in the synthesis of exosomes, which allow proteins and RNA to be sorted to create exosomes with unique biochemical characteristics (45). Exosomes are small (30–100 nm in diameter) and have an endosomal origin. They are discharged by almost all cell types. Primary endocytic vesicles are formed by the invagination of plasma membranes, which fuses to produce early endosomal compartments (46). These compartments grow into multivesicular bodies (MVBs), which are composed of a tiny cytosol without organelles and a lipid bilayer that contains a variety of proteins and nucleic acids (47). Exosomes can also be released by inducible processes or through the trans-Golgi network (48). Extracellular materials and recycled molecules are sorted and directed to different cellular locations by the trans-Golgi network. The regulation of vesicular trafficking, encompassing vesicle budding, transport, and docking/fusion, is significantly influenced by proteins belonging to the Rab family of small GTPases (49).

ctDNA

After a team of French researchers announced the first finding of cell-free nucleic acids in circulation in 1948, many investigations were conducted to try to figure out how DNA fragments from healthy, inflammatory, or sick cells are released into the serum (50). It is thought that both necrosis and apoptosis pathways cause cell death, which is how these DNA fragments end up in the bloodstream. But it's still unknown what precise mechanisms control the active release of DNA fragments into the bloodstream (51). Although this theory is not generally accepted, recent research suggests that tumor cells release microvesicles called exosomes that contain fragments of double-stranded DNA (ctDNA) (3). While circulating cell-free DNA (cfDNA) is present in healthy people, cancer patients have a noticeably higher concentration of it (52). ctDNA is liberated from primary and metastatic tumor sites via the previously mentioned pathways. Cell-free DNA is most likely produced from apoptotic cells and is usually seen as double-stranded pieces of 150–200 base pairs, similar to nucleosome-associated DNA (53, 54). "ctDNA"

refers to the portion of cfDNA generated from tumors (55). Multiple tumors in cancer patients affect the release of ctDNA, which may include genetic changes and mutations comparable to those in the primary tumor. Normal physiological conditions cause necrotic and apoptotic cell debris to accumulate quickly because of enhanced cellular turnover, which is removed by invading phagocytes. As a result, under normal circumstances, there is a marked increase in the release of biological materials, such as ctDNA, from necrotic and apoptotic cells into the circulation. While ctDNA levels in cancer patients may be an indicator of tumor burden, there is a significant degree of heterogeneity among those with comparable cancer types, which may indicate biological differences or variances in the rates of cell death within individual tumors (56). Additionally, the proportion of detectable ctDNA varies significantly amongst individuals with different types of tumors, which adds a significant difficulty to the identification and analysis of ctDNA against a background of normal germline cfDNA.

The Role of CBs in Prognostics, Therapeutics, and Diagnostics
CTCs:

Circulating tumor cells (CTCs) must be isolated in order to help oncologists identify cancer early and customize therapies, which will enable more efficient and individualized care. CTC counting has been shown in recent clinical studies to have potential in prognosis prediction and therapy monitoring for early cancer identification (57, 58). A more comprehensive picture of the course and prognosis of cancer, along with information on therapy sensitivity and resistance, can be obtained by integrating CTC counts with thorough cfDNA genetic characterization (59–61). Several genomic profiling methods can be applied once single CTCs have been identified. Leukocyte contamination can be prevented by analyzing single CTCs, which makes it possible to examine CTC heterogeneity and co-existing mutations. For mutational research, sensitive whole-genome amplification (WGA) is required because a single cell has around 6.6 picograms of DNA (62). Still, separating technical noise from variations in gene expression that are physiologically meaningful is a major task. Ramskold et al. (63) used the Smart-Seq procedure to solve this, which provides decreased technical variance and improved transcriptome coverage over previous mRNA-Seq approaches (64).

Exosomes:

Exosomes are thought of as microscopic copies of their parent cells, with a complex makeup of lipids, proteins, and nucleic acids that are sorted differently depending on the type of cell from which they originate (65). Their intricacy and possible utility can be seen in the 4,400 proteins, 194 lipids, 1,639 mRNAs, and 764 miRNAs that they may include (66, 67). Exosomal contents can reveal particular activation or disease states. For example, Fiandaca et al. reported elevated phosphorylated tau and beta-amyloid proteins in Alzheimer's patients (69), while Shi et al. observed increased alpha-synuclein levels in plasma exosomes from patients with Parkinson's disease (68). Exosomes include functional chemicals that can affect the activities of target cells (70), and exosomal miRNAs have been demonstrated to repress genes downstream, which may contribute to the development of tumors. Exosomes have been shown in numerous studies to have diagnostic and therapeutic value in a range of diseases,

including cancer, neurodegenerative, infectious, and cardiovascular disorders. Exosomes derived from human prostate and breast cancer cell lines, for instance, have consistently demonstrated positive for CD81 and CD9 (72). Furthermore, compared to healthy persons, melanoma patients show considerably increased levels of exosomal melanoma inhibitory activity (MIA) and S100B (73). Exosomal NY-ESO-1 has been significantly associated with worse survival outcomes in non-small cell lung cancer (74). Additionally, exosomes show promise for use in gene silencing, medication delivery, tissue regeneration, and vaccine development.

ctDNA:

In liquid biopsies, circulating tumor DNA (ctDNA) has shown promise as a biomarker for cancer diagnosis and prognosis (81). To genotype point mutations, especially those that are important for therapy choices, ctDNA is frequently used. By representing the genetic makeup of the entire tumor, its analysis lessens the limits of tissue biopsies (56). Increased levels of ctDNA have been linked to tumor burden in patients with breast cancer (82) and have the potential to be used as a diagnostic marker for hepatocellular carcinoma (83). Because of its brief half-life, ctDNA can offer real-time information about alterations in the tumor genome, enabling the prediction of treatment resistance before clinical progression (84). In order to reliably predict metastatic relapse and allow for prompt treatments, Garcia-Murillas et al. (85) created a tailored assay for tracking mutations in patients with early-stage breast cancer after treatment (86, 87). Furthermore, by identifying resistance mutations, ctDNA can be used to evaluate the effectiveness of a treatment (56, 89, 90). When ESR1 mutations are discovered early on in the course of breast cancer, alternative treatments may be initiated, potentially improving results (91). Analogously, KRAS mutations in patients with colorectal cancer may signify resistance to inhibitors of the epidermal growth factor receptor (56).

Isolation and Purification of Circulating Biomarkers

Current technologies for enriching and isolating circulating biomarkers (CBs) exploit their unique biological and physical characteristics, differentiating them from normal blood cells. For circulating tumor cells (CTCs), this includes properties like size, density, deformability, and surface protein expression, primarily EpCAM (92). To effectively study and utilize extracellular vesicles (EVs), precise isolation from a wide array of cellular debris is essential. The most common method for EV isolation is ultracentrifugation, including differential centrifugation, due to its high capacity for processing (93). For circulating tumor DNA (ctDNA), isolation techniques are crucial as ctDNA is finite and can be contaminated by high molecular weight DNA from leukocytes. Various cfDNA extraction kits, such as the QIAamp circulating nucleic acid kit (Qiagen) and NucleoSpin® Plasma XS kit (Macherey-Nagel), utilize silica gel membranes or magnetic beads for efficient extraction (94). Careful consideration of the extraction and mutation detection methods is essential to minimize artifacts (17, 95).

Size-based Enrichment and Isolation Technologies

Size-based methods for CTC separation capitalize on the different geometrical properties between cancer cells and blood cells. These methods typically use membrane microfilters, allowing for viable, label-free cells that can be analyzed downstream, such as through next-generation sequencing (NGS). However, many CTCs are similar in size to normal blood cells, making isolation challenging. The accuCyte-CyteFinder assay addresses this by utilizing density-based separation (96). The CTC iChip technology combines size-based enrichment with either EpCAM-positive selection or CD45-negative depletion, although it is limited to single cells or small clusters (97, 98).

Immunoaffinity-Based Methods

Immunoaffinity approaches for isolating CTCs often involve magnetic bead separation with antibodies targeting epithelial markers. Positive selection typically focuses on EpCAM, while negative selection targets non-expressed antigens, such as CD45 (99, 100). This method involves using magnetic fields to isolate labeled cells post-antigen binding (101). Notably, the FDA-approved CellSearch system captures CTCs based on EpCAM expression, identifying them through positivity for cytokeratins and negativity for CD45 (102). However, challenges such as epithelial-to-mesenchymal transition (EMT) can impact the capture of aggressive CTC subpopulations (103, 104). For exosome isolation, immunoaffinity interactions leverage proteins and receptors on exosome membranes. Studies have shown that antibody-coated magnetic beads can efficiently isolate exosomes, provided appropriate membrane markers are selected (105). Techniques such as ELISA have been developed for exosome quantification from various bodily fluids, demonstrating high specificity (106–108). Other methods, such as HER-2 coated paramagnetic beads, have shown effective isolation of cancer-specific exosomes (109).

Microfluidic-Based Enrichment Technologies

Microfluidic devices facilitate precise fluid flow control, enhancing cell capture efficiency. The CTC-chip, featuring microposts functionalized with anti-EpCAM antibodies, enriches CTCs from blood samples (110). The cluster chip captures CTCs via bifurcating traps and flow reversal (111), while the herringbone-chip (HB-Chip) serves as a high-throughput isolation platform (112). The Ephesia CTC-chip utilizes magnetic traps for advanced cancer cell testing (113). Acoustophoresis offers a label-free method based on size, density, and compressibility, utilizing acoustic standing waves for separation. Microfluidic technologies are now at the forefront of exosome isolation, providing advantages like minimal processing time, high throughput, and sensitivity. Methods can be divided into immunoaffinity-based isolation and those using acoustic waves or dielectric electrophoresis (115, 116). These advancements underscore the potential of microfluidic systems in enhancing the efficiency and accuracy of CTC and exosome detection and analysis.

Main Role of Radiologist

Radiologists play a crucial role in the multidisciplinary approach to early cancer detection and management. By utilizing advanced imaging techniques such as

PET scans and MRI, radiologists provide essential insights into tumor characteristics, including location, size, and metabolic activity. Their expertise in interpreting these images complements the data obtained from liquid biopsies, offering a comprehensive diagnostic picture. This collaborative effort between liquid biopsy technologies and radiology ensures accurate diagnosis, effective treatment planning, and better patient outcomes, particularly in complex or atypical cases of cancer.

Conclusion

Liquid biopsy represents a revolutionary shift in cancer diagnostics and monitoring, addressing the shortcomings associated with traditional tissue biopsies. By analyzing circulating biomarkers such as CTCs, ctDNA, and exosomes, liquid biopsies facilitate non-invasive, real-time insights into tumor genetics, allowing for timely detection of treatment responses and early identification of therapeutic resistance. Radiological imaging, including PET scans and MRI, complements these insights by providing detailed information on tumor location, size, and metabolic activity. This innovation is particularly significant in the context of personalized medicine, where understanding tumor heterogeneity and dynamics is critical for tailoring effective treatment strategies. As the demand for precision oncology increases, integrating circulating tumor biomarkers and advanced imaging techniques into routine clinical practice becomes imperative. Although current technologies, such as CellSearch® for CTC analysis, have gained FDA approval, challenges remain, particularly concerning the heterogeneity of CTCs and the need for improved isolation and detection methodologies. Advancements in microfluidics, size-based separation techniques, immunoaffinity methods, and imaging modalities promise to enhance the efficiency and specificity of cancer diagnostics. Future research must focus on validating the clinical utility of these biomarkers and imaging techniques through large-scale, multicenter studies. Such efforts will provide the robust evidence required for widespread adoption in clinical settings, ultimately improving patient outcomes. The promise of liquid biopsy and radiological imaging technologies lies not only in their ability to transform cancer care but also in their potential to usher in a new era of personalized, less invasive diagnostics and treatment strategies tailored to individual patient needs.

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