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Review Article

Role of Extracellular Vesicles and Exosomes in Liquid Biopsy: Novel Biomarkers for Non-Invasive Disease Detection

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Abstract

Thanks to liquid biopsy, it is now possible to diagnose, predict and monitor the treatment strategy based on the analysis of components of body fluids. Scientists have examined many biomarkers lately, but the modern-day exosomes are proving to be very popular due to their stable carriage. This load is also physiologically active. Cargo includes proteins, lipids, mRNAs, microRNAs, and DNA fragments, among others. As such, because they reflect the molecular characteristics of the cell of origin closely, they are excellent sources of diagnostic and prognostic markers. The article reviews the study of biological properties of extracellular vesicles and exosomes, their biogenesis, composition, separation, and characterisation. The article explains the biomarkets industry and various applications of liquid biopsy such as detection of diseases. Different markers are explored for their ability to detect disease early, monitor the progress, and guide therapy. Despite their promise, issues remain, including uneven approaches to isolation, unstandardized protocols, and minimal clinical validation. With the advancement of bioinformatics and molecular technologies, the clinical utility of exosome-based diagnostics will likely improve and become an important tool of precision medicine.

1. Introduction

Liquid biopsy is now being thought of as a possible alternative to tissue biopsy as, there is an increasing demand for early, accurate, least invasive diagnosis. Tissue biopsy remains the gold standard for pathological diagnosis. However, it has many disadvantages such as being invasive and incurring sample error. Additionally, it does not offer real-time information regarding the evolution of the disease. The molecular analysis of circulating components, often referred to as circulating tumor DNA, circulating tumor cells and extracellular vesicles, found in body fluids is made possible by liquid biopsy and provides vital information for monitoring and diagnosing the disease [1]. Extracellular vesicles (EVs) are significant mediators of intercellular communication and potential sources of therapeutic biomarkers. Various cell types release these membrane-bound vesicles that contain a mixture of biological components such as proteins, lipids, messenger RNAs, microRNAs, and DNA fragments. The composition of EV cargo varies depending upon physiological or pathological conditions as it reflects the molecular characteristics of the parent cells [2]. Due to their stability in body fluids and ability to shuttler useful molecular cargo between cells, exosomes, a main subtype of extracellular vesicles with particle size range of about 30–150 nm, have received enormous attention. Exosomes produced by diseased cells, particularly tumor cells, are promising candidates for non-invasive diagnosis, prognosis prediction, and therapy monitoring because they carry distinct molecular signatures that can be detected in blood and other body fluids [1]. While extracellular vesicles and exosomes hold great potential as liquid biopsy markers, there are still some hurdles to their widespread use

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in the clinic. They are the unavailability of internationally-accepted standards and differences in characterisation approaches and isolation methods. Recent recommendations MISEV2023 continue and new strategy of producing, separating, characterizing EV aimed at improving the clinical translation, reproducibility, and reliability of EV studies [2].

2. Biological Characteristics and Biogenesis of Extracellular Vesicles and Exosomes

Almost all cell types secrete little membrane-enclosed, nanoscale items called extracellular vesicles into the extracellular environment. They are crucial to cell-to-cell communication via transference of biological information from donor to recipient cells. Exosomes, microvesicles, and apoptotic bodies are three main types of EVs classified according to their biogenesis mechanisms, size and molecular characteristics. As a result of their biological function, stability, and the application potential in clinical diagnostics, exosome is the vesicle subtype that has received most attention [3]. Exosomes are small extracellular vesicles that originate in the endosomal system. Their diameter is generally between 30 and 150 nm. In multivesicular bodies, endosomal membrane invagination produces intraluminal vesicles. When these multivesicular structures fuse with the plasma membrane, they then release exosomes into the extracellular environment. The endosomal sorting complex required for transport (ESCRT) pathway and ESCRT-independent processes are two of the molecular mechanisms involved in this tightly controlled process [4]. Proteins, lipids, nucleic acids, and other bioactive compounds make up the molecular cargo of exosomes, which primarily determines their biological roles.

Exosomes carry mRNA, microRNAs, long non-coding RNAs, and DNA fragments. They can affect gene expression and cellular behaviour of recipient cells. The cargo of exosomes reflects the molecular state of the parent cells [5], and therefore, such vesicles provide valuable information about normal as well disease processes. Release and uptake of extracellular vesicles modulate many physiological and pathological processes such as immune modulation, tissue regeneration, inflammation, tumour growth and metastasis. Exosomes produced by tumors can modify the environment surrounding tumors, encourage the formation of blood vessels (angiogenesis), help tumors avoid the immune system, and prepare remote areas for the spread of metastases.

It is these characteristics that have led exosomes to receive increasing attention as potential biomarkers for early disease detection and monitoring [6] as shows in Figure 1.

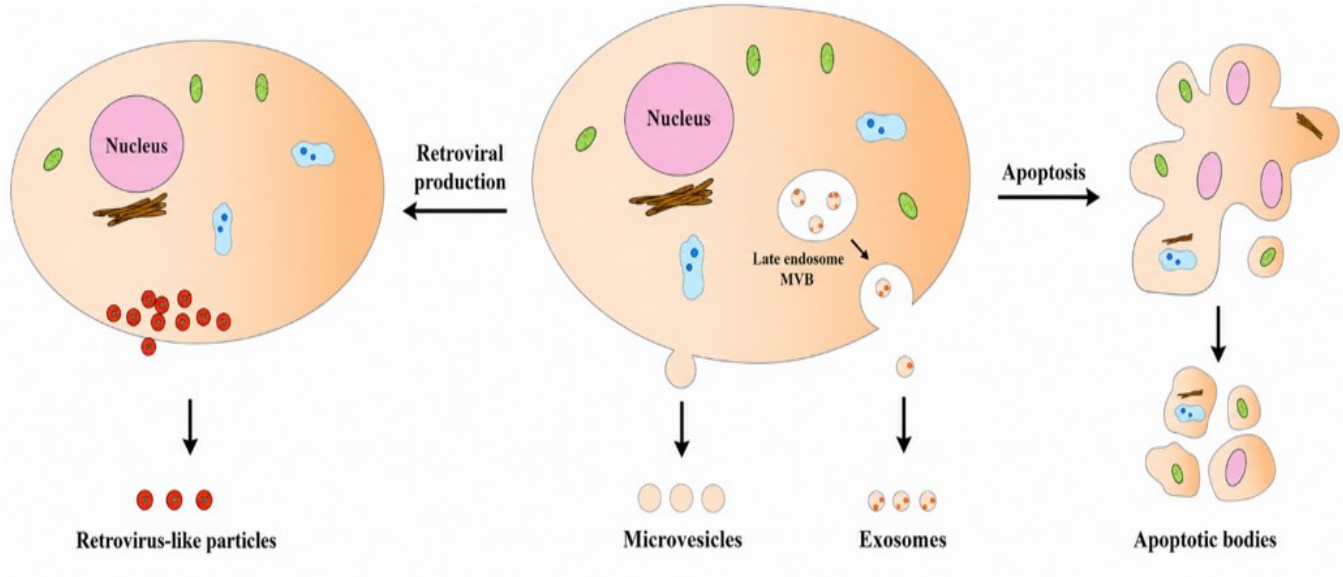

Figure 1: Biogenesis and classification of extracellular vesicles including exosomes, microvesicles, and apoptotic bodies [7]

3. Molecular Cargo of Exosomes: Proteins, Lipids, and Nucleic Acids as Diagnostic Biomarkers

Complex molecular cargo of exosomes is made up of proteins, lipids, nucleic acids and other biomolecules that represents the physiological state of the parent cells. Molecules that are selectively packaged into exosomes can serve as biological information transmitters between cells. Exosomal cargo can be protected from enzyme degradation because the surrounding lipid bilayer membrane. As a result, these molecules can serve as stable and readily accessible biomarkers for illness detection using liquid biopsy approaches. Exosomal proteins are one of the most studied molecular components for diagnosis. These proteins are commonly known exosomal markers like tetraspanins CD9, CD63 and CD81 along with heat shock proteins, and membrane proteins involved in vesicle formation and cell communication. In addition, exosomal disease-specific proteins may provide information about the modifications in pathology. a tumor to secrete these vesicles and there are studies to show that these vesicles are capable of triggering tumor progression, invasion and immune modulation [4].

If so, exosome-associated proteins may have value as diagnostic and prognostic biomarkers for cancer [8]. Besides proteins, the lipids are also significant for exosomal structure and functions. The membranes of exosomes are composed of cholesterol, sphingomyelin, phosphatidylserine, and various other lipid molecules which stabilize the vesicle and shield its cargo. Exosomal lipid composition alterations are linked with sickness development and cellular stress responses. As a result, the lipid profiling of extracellular vesicles may provide a new avenue towards the identification of disease-associated molecular signatures [9]. Amongst exosomal molecules, nucleic acids (especially microRNAs (miRNAs)) have attracted much attention as promising diagnostic biomarkers. Exosomal miRNAs are protected from degradation and can regulate gene expression in receiving cells by influencing multiple cellular pathways. The expression patterns of some exosomal miRNAs are altered in various diseases, especially cancers, where they may reveal the tumour initiation, progression, metastasis

and therapy response. According to recent research, monitoring and analyzing exosomes might prove beneficial for diagnosis and prognosis of numerous disorders such as cancer, cardiovascular disease, neurodegenerative diseases, and more. This makes exosomal miRNA analysis a powerful strategy in liquid biopsy-based diagnostics [10]. Similarly, recent advances in sequencing technologies and molecular analysis platforms have expanded the ability to characterize exosomal cargo at high resolution. The use of next-generation sequencing, quantitative PCR, proteomics, and lipidomics made it possible to identify disease-specific exosomal signatures. These findings promote the development of exosome-based liquid biopsy assays for early diagnosis, disease monitoring and personalised medicine [1]. To highlight the diagnostic merits of EVs, Table 1 compares the major liquid biopsy analytes on key diagnostic aspects.

Table 1: Comparison of major liquid biopsy analytes

Feature	**Exosomes (EVs)**	**Circulating Tumor DNA (ctDNA)**	**Circulating Tumor Cells (CTCs)**
Stability in body fluids	High; protected by a lipid bilayer, making them resistant to enzymatic degradation	Low to moderate; susceptible to nuclease degradation	Variable; intact cells are fragile during collection and isolation
Abundance	Very high (10^9–10^{11} particles/mL in biological fluids)	Low to moderate; represents only a fraction of total cell-free DNA	Very low; rare cells in peripheral blood
Molecular cargo	Proteins, lipids, mRNA, miRNA, lncRNA, circRNA, DNA, metabolites	Genomic and epigenomic DNA fragments	Whole viable cells containing DNA, RNA, proteins, and organelles
Main clinical advantage	Reflects real-time intercellular communication and tissue-specific origin	Sensitive detection of tumor-specific genetic mutations	Enables functional cellular analysis and single-cell characterization
Sensitivity for early detection	High, owing to abundance and molecular diversity	Moderate; limited by low ctDNA concentration in early-stage disease	Low because of the rarity of circulating tumor cells
Typical detection methods	Ultracentrifugation, size-exclusion chromatography, immunoaffinity capture, microfluidics	qPCR, digital PCR (ddPCR), next-generation sequencing (NGS)	Immunomagnetic enrichment, filtration, density-gradient centrifugation, microfluidics
Major clinical applications	Disease diagnosis, prognosis, treatment monitoring, biomarker discovery, therapeutic delivery	Mutation profiling, minimal residual disease (MRD), treatment response monitoring	Metastasis assessment, drug susceptibility testing, personalized medicine
Main limitations	Isolation methods remain labor-intensive and lack standardization; vesicle heterogeneity	Low abundance in early disease and fragmented DNA limits biological information	Low recovery rate, difficult isolation, poor reproducibility, high cost
Overall clinical potential	Highly promising for precision medicine due to stability and comprehensive molecular information	Widely adopted in oncology for genomic monitoring	Valuable complementary biomarker but limited by low sensitivity and technical challenges

4. Isolation and Characterization Techniques of Extracellular Vesicles and Exosomes

The precise isolation and characterization of extracellular vesicles (EVs) are required steps for their use as authentic biomarkers in liquid biopsy. Acquiring highly pure extracellular vesicles is a technical challenge that arises due to the complex composition of biological fluids such as proteins, lipoproteins and other vesicular structures. The methods of isolation utilized can have a great impact on the yield, purity and molecular composition of the isolated vesicles which may impact subsequent diagnostic assays [11].

Isolation techniques have been introduced for the extraction of the extracellular vesicles, with differential ultracentrifugation being one of the most utilised methods.

Sequentially pelleting and resuspending vesicles through centrifugation based on size and density. Ultracentrifugation is widely used in research laboratories, but it has certain limitations, including long processing time, potential damage to vesicles and co-isolation of non-vesicular contaminants.

Other strategies have been devised to enhance purity and efficiency [12]: size-exclusion chromatography, polymer-based precipitation, and immunoaffinity-based isolation. After isolation, thorough biochemical and biophysical characterization is obligatory to confirm vesicle identity and purity according to standard criteria. Through the use of nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM), processes that check for particle size distribution, concentration and structural morphology are routinely performed on all exosomal preparations. Furthermore, the enrichment of specific surface markers (e.g. tetraspanins CD9, CD63, and CD81) is usually confirmed by way of immunoblotting or flow cytometry [13]. Such thorough characterization will ensure that downstream analyses accurately reflect genuine exosomal signals instead of merely co-isolated protein aggregates and non-vesicular contaminants. The molecular characterization of exosomal cargo has become a key focus for the discovery of disease-related biomarkers [13].

Advanced methods such as quantitative PCR (qPCR), next-generation sequencing (NGS), mass spectrometry and proteomic analysis allow for the detection and analysis of specific nucleic acids and proteins carried by exosomes. The discovery of exosomal microRNAs, mRNAs, and protein signatures has been made possible by these approaches which may serve as diagnostic and prognosis biomarkers in diseases [1]. However, despite significant technologic advances, the unavailability of standardized protocols for extracellular vesicle isolation and characterization remains a major hurdle for their clinical translation. Changes in sample preparation, isolation methods, and analytical platforms can lead to differences in reported results between studies. The most recent recommendations, MISEV2023, advocate for standard operating procedures and validation strategies, to ensure reproducibility in disease diagnosis through platforms based on EVs [3] as shows in Figure 2.

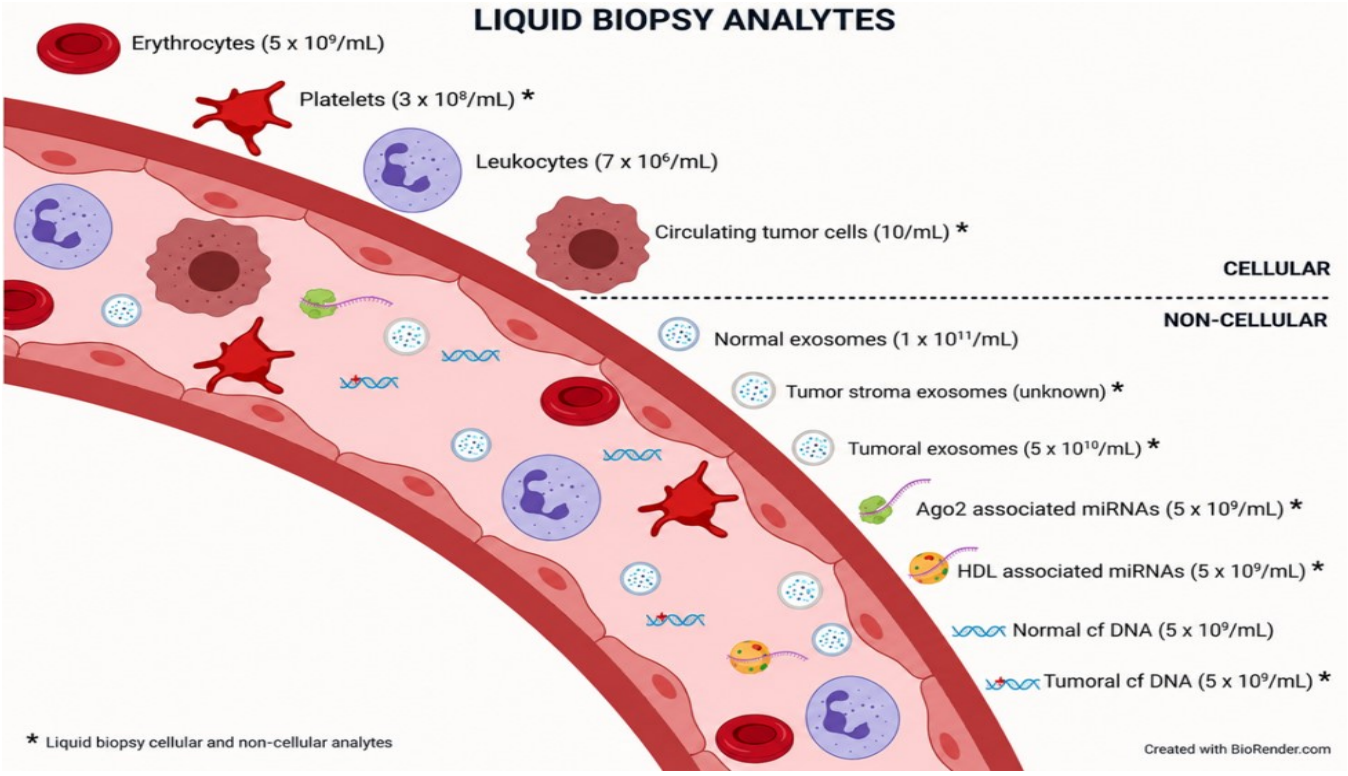

Figure 2: Workflow of exosome-based liquid biopsy from sample collection to clinical biomarker identification [14]

5. Clinical Applications of Extracellular Vesicles and Exosomes in Liquid Biopsy

5.1. Cancer Diagnosis and Prognosis

One of the most common applications of extracellular vesicles and exosomes for liquid biopsy in clinic is cancer. The release of exosomes containing tumor-associated molecules is characteristic of many cancers. Such molecules include proteins, oncogenic microRNAs, mRNA, and even DNA. Exosomes from tumor cells are found in biological fluids and provide indications of initiation, progression, metastasis, and response to therapy. Compared to traditional tissue biopsy, exosome-utilizing liquid biopsy is a safer method for the repetitive monitoring of the tumour [1]. Furthermore, exosomal biomarkers have demonstrated remarkable diagnostic potentials in various cancers. For example, altered expression profiles of miR-21, miR-155, and miR-1246 are associated with tumor development and tumour progression. Moreover, in a few cancers, i.e. breast cancer, lung cancer, colorectal cancer, and pancreatic cancer the proteins and surface molecules of exosomes are studied as diagnostic and prognostic biomarkers. Research shows that the molecular profile of exosomes can serve as a non-invasive biomarker for precision oncology [15] Figure 3. Disease profiling through analysis of exosomal molecular cargo along with cell-free DNA is a useful diagnosis.

5.2. Cardiovascular Diseases

The development of CVD mainly the role of extracellular vesicles and exosomes are receiving extensive use as biomarkers for cardiac injury and cardiac diseases. Cardiomyocytes, endothelial cells, and immune cells all secrete EVs that contain distinct molecular signals that are involved in inflammation, endothelial dysfunction, and myocardial injury. Changes to the amount and molecular composition of circulating EVs have been associated with myocardial infarction (heart attack), heart failure and atherosclerosis [17]. Exosomal microRNAs (miRs), like miR-1, miR-133a and miR-208a, have been investigated as candidates for cardiac injury detection as they regulate cardiac muscle and cardiac stress. This paper presents an overview of osteoporosis in cardiovascular diseases worldwide.

As well as this, it also highlights the increasing burden of diseases and treatment [18].

5.3. Neurological Disorders

Extracellular vesicles have attracted increasing interest for therapeutic application in neurological diseases as exosomes can cross the blood brain barrier and carry molecular cargo from neural cells. Because of this property, they are good candidates for detecting neurologic changes in body fluids. Research has been conducted on the exosomal biomarkers of neurodegenerative diseases which include Alzheimer's Disease, Parkinson's Disease, and Amyotrophic lateral sclerosis [19]. Certain exosomal proteins and nucleic acids such as phosphorylated tau protein and disease-associated microRNAs may be able to identify pathological processes occurring in the CNS. This liquid biopsy method based on exosomes can be a less invasive strategy for the diagnosis and monitoring of neurological diseases [20].

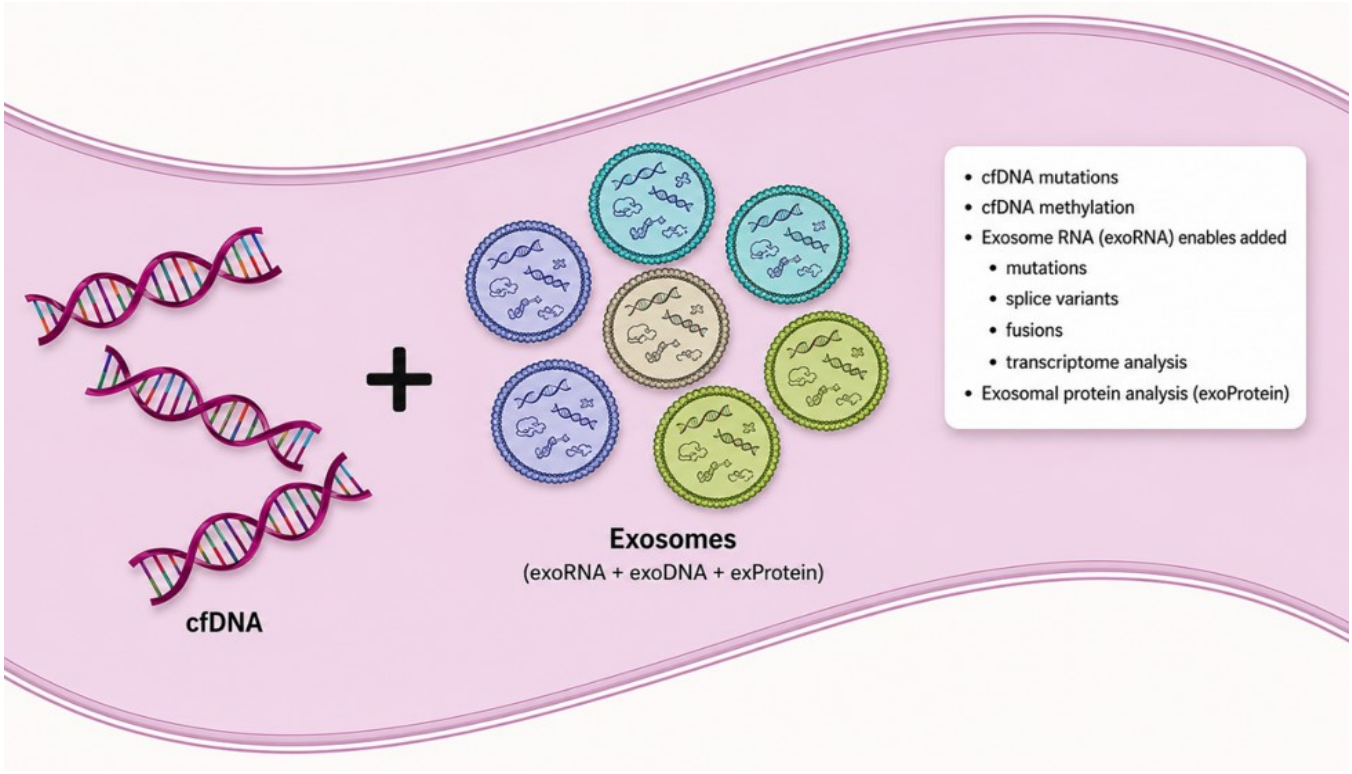

Figure 3: Clinical applications of extracellular vesicles and exosomes as liquid biopsy biomarkers [16]

5.4. Infectious Diseases

Because pathogens and infected cells are capable of modulating EV production and cargo composition, EVs have been explored as biomarkers in infectious disease. An important advantage of exosomes may be detectable during infection. These may contain pathogen-derived molecules, including nucleic acids and proteins. The technique has promise viral bacterial and parasitic infections [21]. Exosomal biomarkers have been researched in viral infections such as hepatitis and Covid-19, in which changes in the EV content might reflect immune response and disease severity. Liquid biopsy on EV may help in early detection and provide critical information on host–pathogen interactions [4] as shows in Table 2.

Table 2: Examples of Exosomal Biomarkers in Different Diseases [22]

Disease Category	Example of Exosomal Biomarkers	Source of Exosomes	Clinical Application
Cancer	• miR-21, miR-1246, miR-155 • Glypican-1 (GPC1) • Epidermal growth factor receptor (EGFR) • Mutant KRAS mRNA	Tumor cells, tumor microenvironment, immune cells	• Early diagnosis • Prognosis prediction • Monitoring disease progression • Prediction of therapy response and resistance
Cardiovascular Diseases	• miR-208a, miR-499, miR-1 • miR-133a, miR-126 • Cardiac troponin I (cTnI) • Heat shock protein 60 (HSP60)	Cardiomyocytes, endothelial cells, platelets, immune cells	• Detection of myocardial injury • Diagnosis of acute myocardial infarction • Heart failure monitoring • Risk assessment in atherosclerosis
Neurological Diseases	• Phosphorylated tau (p-tau) • α-Synuclein • miR-132, miR-124, miR-9 • Amyloid-β (Aβ)	Neurons, astrocytes, microglia, neural stem cells	• Early diagnosis of neurodegenerative diseases • Disease progression monitoring • Differential diagnosis • Evaluation of therapeutic response
Infectious Diseases	• Pathogen-derived RNA/DNA • Viral proteins (e.g., N protein, E protein) • miR-146a, miR-155 • Bacterial components (e.g., LPS)	Infected cells, immune cells, pathogens	• Early detection of infection • Monitoring disease severity • Understanding host-pathogen interactions • Guidance for treatment strategies

6. Challenges and Future Perspectives of Exosome-Based Liquid Biopsy

Although extracellular vesicles and exosomes have great potential as liquid biopsy biomarkers, problems hinder their widespread clinical use. It is the lack of standards and universally accepted protocols for sampling, cell-free vesicle extraction, and characterization is one of the key hurdles. The impact on quantity and quality of resultant vesicles may differ due to variations in isolation approaches including ultracentrifugation, precipitation methods, size-based separation methods, etc [3]. Another major challenge is the complexity of extracellular vesicle populations in biological fluids. Body fluids contain mixed populations of vesicles from different cell types and consequently, it is not easy to separate out those associated with disease from those that are being produced normally by the body and thus are physiological. To enhance the specificity of exosome detection, sophisticated technologies are required such as those that identify unique molecular signatures and reliably associate vesicles with their cellular origins [11]. Furthermore, the successful translation of exosome-based biomarkers from research laboratories to clinical laboratories will also require large-scale validation studies and the establishment of reliable diagnostic platforms. Many studies have identified potential biomarkers in exosomes, however, only a few have made it to clinical use due to issues in regulatory approval and sensitivity, specificity, and reproducibility issues.

Standardised analytical workflows and multicentre clinical trials will be important to verify the diagnostic potential of EV-based assays [23].

Future progress in extracellular vesicle research will enhance the clinical utility of liquid biopsy strategies. Advanced technologies like Next Generation Sequencing, artificial intelligence, nanotechnology, and High throughput molecular profiling can facilitate the detection of disease associated exosomal signatures. These advances may allow for more precise diagnosis, individualized therapeutic strategies and the real-time monitoring of disease progression [1].

Overall, extracellular vesicles and exosomes represent a fast-growing field with enormous potential to modern diagnostics. Due to their capacity to transport stable molecular information from diseased cells and their presence in accessible biological fluids, they are attractive candidates for next-generation non-invasive biomarkers. To successfully implement these methods in regular medical diagnostics, it is necessary to carry out continuing progress in terms of isolation methods, characterization techniques as well as clinical validation [24].

7. Integrative Bioinformatics and Artificial Intelligence in Exosome Analysis

The large amount of data generated from exosomal multi-omics-filings like next-generation sequencing and mass spectrometry will require sophisticated computational frameworks [25]. Artificial Intelligence (AI) and machine learning (ML) algorithms have become a useful means to parse heterogeneous exosomal signatures [26]. ML models trained on clinical metadata and exosomal RNA and protein profiles will recognize subtle molecular signatures predicting early localization versus later-stage disease, dramatically improving accuracy and predictive power. The future of clinical translation will depend on validated AI-based bioinformatics pipelines that automate data interpretation and reduce technical variations across multi-centre cohorts [27].

8. Conclusion

The importance of extracellular vesicles and exosomes as liquid biopsy components stems from their ability to maintain stable, cell-specific, and disease-specific cargo as they move from their cell of origin. Due to their distinct biological properties; including high abundance in bodily fluids, protective function towards molecular cargo, and involvement in intercellular communication they are potential candidates for non-invasive diagnostic and prognostic biomarkers.

Through the examination of exosomal proteins, lipids, microRNAs and other nucleic acids, a significant potential has been demonstrated to detect and monitor diseases like cancer, cardiovascular disorders, neurological diseases, infectious diseases and so on. Technological advancements in molecular biology have further improved the characterization of exosomes and their clinical applications.

To be able to adopt exosome-based biomarkers as regularly incorporated clinical practice, several obstacles have to be overcome. These obstacles include the lack of standardised isolation protocols, technical variability as well as large scale clinical validation. Future studies that utilise advanced analytics analysis tools and AI with nanotechnology could spearhead the translational pathway of experimental studies of Extracellular Vesicle-based liquid biopsy to reliable clinical diagnosis.

All in all, extracellular vesicles and exosomes comprise a new generation of biomarkers that are minimally invasive and can greatly help the early detection, prognosis assessment and personalized medicine. Further research and technological advancement will be critical for the full potential of these for the future of precision diagnostics.

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Data Availability: Data availability is not applicable to this article because no datasets were generated or analyzed.
Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

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