STM JOURNALS

Research & Reviews: Journal of
Oncology and Hematology

ISSN: 2319-3387
Volume 15, Issue 1, 2026
January–April
DOI (Journal): 10.37591/RRJoOH

https://journals.stmjournals.com/rrjooh

Review RRJoOH

Extracellular Vesicle-Based Liquid Biopsies: Decoding the Tumor Microenvironment for Precision Oncology

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Abstract

The tumor microenvironment (TME) plays a pivotal role in cancer initiation, progression, and therapeutic response. Decoding the TME is, therefore, essential for advancing precision oncology. Extracellular vesicles (EVs), including exosomes and microvesicles, are nanoscale lipid bilayer particles secreted by tumor and stromal cells. They transport a wide range of bioactive molecules, such as DNA, RNA, proteins, lipids, and metabolites, which reflect the dynamic state of the TME. Recent advances in liquid biopsy have highlighted EVs as promising, minimally invasive biomarkers for cancer diagnosis, prognosis, and therapy monitoring. Unlike traditional tissue biopsies, EV-based liquid biopsies provide a real-time snapshot of tumor biology and can capture spatial and temporal heterogeneity. In this review, we critically evaluate the role of EV-derived biomarkers in immune modulation, angiogenesis, metastasis, and therapy resistance within the TME. We also summarize emerging EV isolation and characterization technologies, ranging from ultracentrifugation to microfluidic and nano-plasmonic platforms. Current challenges are addressed, including assay standardization, vesicle heterogeneity, and clinical validation. Looking ahead, the integration of EV-based liquid biopsy with multi-omics profiling and artificial intelligence has the potential to accelerate translation into clinical oncology. By bridging molecular pathology with translational research, EV-derived biomarkers may provide powerful tools for early detection, patient stratification, and precision-guided therapies. This review emphasizes the transformative potential of EV-based liquid biopsies in decoding the TME and shaping the future of personalized cancer management.

Keywords: Cancer biomarkers, exosomes, extracellular vesicles, immune modulation, liquid biopsy, metastasis, microfluidics, multiomics, nano-diagnostics, precision oncology, therapy resistance, tumor microenvironment

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Received Date: January 21, 2026
Accepted Date: February 04, 2026
Published Date: April 05, 2026

Citation: Shubham Sah, Deepa Kumari, Manish Kumar, Meghana Inupamula, Abhay Kumar Yadav, Mohan Mishra, Abhijeet Pandey. Extracellular Vesicle-Based Liquid Biopsies: Decoding the Tumor Microenvironment for Precision Oncology. Research & Reviews: Journal of Oncology and Hematology. 2026; 15(1): 43–62p.

INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide, accounting for millions of deaths annually despite advances in screening, diagnosis, and treatment strategies [1–3]. Cancer is increasingly recognized not as a disease of malignant cells alone, but rather a highly dynamic and multifactorial process shaped by the interaction between tumor cells and their surrounding biological environment [4]. Modern oncology has thus shifted from a tumor cell-centric view to a systems-level perspective that integrates molecular, cellular, and extracellular factors driving disease progression [5]. This evolving understanding has fueled the development of innovative diagnostic and therapeutic tools to improve patient outcomes by enabling early

detection, real-time disease monitoring, and personalized treatment [6]. Among the most transformative concepts in this regard are liquid biopsy, the tumor microenvironment (TME), and extracellular vesicles (EVs), each offering unique insight into cancer biology while being deeply interconnected. Traditional diagnostic approaches in oncology have largely relied on tissue biopsy, histopathology, and imaging to characterize tumors and guide treatment decisions [7]. These methods are limited by their invasiveness, inability to capture tumor heterogeneity, and unsuitability for repeated longitudinal monitoring.

In certain clinical scenarios – such as inaccessible tumor sites, advanced disease stages, or frail patients – obtaining tissue biopsies may not be feasible [8]. These limitations have accelerated the search for alternative approaches, leading to the emergence of liquid biopsy as a minimally invasive yet highly informative diagnostic tool [9]. Liquid biopsy involves the analysis of tumor-derived material in easily accessible body fluids such as blood, urine, saliva, or cerebrospinal fluid [9, 10]. The most commonly studied analytes include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and extracellular vesicles (EVs), each reflecting complementary aspects of tumor biology [11]. By enabling early cancer detection, identifying minimal residual disease, monitoring treatment responses, and capturing the emergence of resistance mutations, liquid biopsy has revolutionized oncology and become an indispensable component of precision medicine [12].

Advances in tumor biology have revealed that cancer development and progression cannot be understood in isolation from the tumor microenvironment (TME) [4]. Far from being a passive backdrop, the TME is a complex and dynamic ecosystem composed of cancer-associated fibroblasts (CAFs), endothelial cells, immune cells, extracellular matrix (ECM) components, and myriad cytokines, chemokines, and growth factors [13]. These components collectively influence virtually every step of tumorigenesis, from the initiation of malignant transformation to local invasion and distant metastasis [14]. TME provides structural support and survival signals to cancer cells while contributing to pathological processes such as angiogenesis, immune suppression, and metabolic reprogramming [15]. The TME can act as a sanctuary that protects tumor cells from chemotherapy, radiotherapy, and even targeted therapies, thereby promoting therapeutic resistance [16]. Recent therapeutic strategies increasingly seek to reprogram or disrupt the TME to restore anti-tumor immunity and enhance treatment efficacy, as seen with immune checkpoint inhibitors, anti-angiogenic drugs, and stromal-targeted interventions [17]. A fascinating area of convergence between liquid biopsy and TME research lies in the study of extracellular vesicles (EVs) [18].

These nanosized, membrane-bound particles – including exosomes, microvesicles, and apoptotic bodies – are secreted by virtually all cell types and are abundant in circulation [19]. Once considered mere cellular waste, EVs are recognized as central intercellular communication mediators [20]. They encapsulate a diverse cargo of nucleic acids (DNA, mRNA, microRNA, long noncoding RNA), proteins, lipids, and metabolites that mirror the physiological or pathological state of their cell of origin. The lipid bilayer membrane surrounding EVs protects their cargo from enzymatic degradation, conferring remarkable stability and making them ideal biomarker reservoirs [21]. Importantly, tumor-derived EVs reflect the molecular signatures of cancer cells and actively shape the TME by promoting angiogenesis, modulating immune responses, and preparing pre-metastatic niches [22]. As biomarkers and functional mediators of disease, this dual role underscores the transformative potential of EVs in oncology [23].

The clinical significance of EVs as biomarker reservoirs is rapidly gaining traction. Because they are detectable in virtually all body fluids, EVs represent a powerful platform for non-invasive diagnostics [24]. In oncology, tumor-derived EVs have been shown to carry oncogenic mutations, such as EGFR, KRAS, and TP53, as well as specific protein and microRNA signatures that correlate with tumor burden, stage, and therapeutic response [25]. Beyond cancer, EVs also hold promise in neurodegenerative disorders, cardiovascular diseases, and infectious diseases, highlighting their broad applicability in precision medicine [26]. Within the TME, EVs facilitate bidirectional communication between tumor cells and stromal or immune cells, often promoting tumor progression by enhancing

angiogenesis, suppressing cytotoxic T-cell activity, and fostering immunosuppressive niches [27]. From a therapeutic perspective, the ability to harness or engineer EVs as delivery vehicles for drugs, RNA molecules, or immunomodulators further expands their relevance beyond diagnostics [28]. These advances illustrate a profound paradigm shift in oncology.

Integrating liquid biopsy, TME dynamics, and EV biology provides a more holistic understanding of cancer as a disease not confined to malignant cells but shaped by continuous molecular and cellular interactions within the host [29]. Liquid biopsy offers a minimally invasive window into tumor evolution; the TME reveals the contextual forces that drive progression and resistance; EVs serve as both messengers and measurable indicators of these processes [30]. The convergence of these fields has far-reaching implications for cancer diagnosis, prognosis, and therapy, paving the way toward truly individualized treatment strategies [31].

This review aims to synthesize current knowledge on these interconnected themes by exploring the evolving role of liquid biopsy in modern oncology, highlighting the significance of the TME in shaping tumor behavior and therapeutic outcomes, and discussing the emerging importance of EVs as biomarker reservoirs and mediators of disease [32]. By examining these concepts together rather than in isolation, we aim to provide a comprehensive perspective on how they collectively advance the field of precision oncology and open new avenues for translational research and clinical application [33].

THE TUMOR MICROENVIRONMENT: A SOURCE OF BIOMARKER COMPLEXITY

Cancer is a multifactorial disease driven by genetic and epigenetic alterations within tumor cells and the dynamic interplay between malignant cells and their surrounding microenvironment [34]. The tumor microenvironment (TME) concept has emerged as a central paradigm in modern oncology, recognizing that tumors exist within a complex ecosystem that actively influences cancer initiation, progression, metastasis, and response to therapy [13]. The TME comprises diverse cellular and non-cellular components, engages in sophisticated intercellular communication, and generates a broad spectrum of molecular and cellular signatures that can serve as biomarkers [35]. These biomarkers provide critical insights into tumor behavior, prognostication, and therapeutic responsiveness, but they also introduce a high degree of complexity due to spatial, temporal, and functional heterogeneity [36]. Therefore, understanding the TME as both a driver of cancer progression and a reservoir of biomarkers is essential for advancing precision oncology and developing clinically actionable strategies [37].

The cellular components of the TME represent a diverse and dynamic network of cells that interact closely with tumor cells to modulate tumor growth, invasion, metastasis, and immune evasion [38]. Cancer-associated fibroblasts (CAFs) are among the most abundant and influential stromal cell types [39]. CAFs are highly heterogeneous and can be identified by markers such as fibroblast activation protein (FAP), alpha-smooth muscle actin (α-SMA), and platelet-derived growth factor receptor (PDGFR) [40]. They secrete a range of growth factors, cytokines, and extracellular matrix-modifying enzymes, including transforming growth factor-beta (TGF-β), hepatocyte growth factor (HGF), and matrix metalloproteinases (MMPs), which promote tumor cell proliferation, invasion, and angiogenesis [41]. CAFs also modulate immune cell recruitment and function, contributing to the establishment of immunosuppressive niches that hinder anti-tumor immunity [42]. The functional diversity of CAFs underscores their role as facilitators of cancer progression and potential sources of prognostic and predictive biomarkers [43]. Tumor-associated macrophages (TAMs) are another critical cellular component of the TME [44].

TAMs often adopt an M2-like phenotype that promotes tissue remodeling, angiogenesis, and immune suppression [45]. High infiltration of TAMs in the tumor microenvironment correlates with poor prognosis in various cancers, including breast, ovarian, pancreatic, and lung cancers [46]. TAMs secrete cytokines, such as interleukin-10 (IL-10) and TGF-β, suppressing cytotoxic T-cell activity and enhancing regulatory T-cell (Treg) expansion [47]. Surface markers, such as CD163, CD206, and CD204, are commonly used to identify pro-tumorigenic TAM populations [48]. In addition to serving

as biomarkers, TAMs are being explored as therapeutic targets, with strategies aimed at reprogramming their phenotype or inhibiting their recruitment to tumors. T lymphocytes, particularly CD8+ cytotoxic T cells, play a central role in anti-tumor immunity, and their presence within the TME is often associated with improved patient outcomes. However, the TME frequently induces T-cell exhaustion through chronic antigen exposure and expression of inhibitory immune checkpoints, including PD-1, CTLA-4, LAG-3, and TIM-3 [49]. Regulatory T cells (Tregs) further contribute to immune suppression by producing inhibitory cytokines such as IL-10 and TGF-β. The balance between effector and suppressive immune cells within the TME – the so-called "immune contexture" – has emerged as a critical biomarker for predicting response to immunotherapies, particularly immune checkpoint inhibitors [50]. Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that expand under chronic inflammation and tumor-derived signals, exerting potent immunosuppressive effects by inhibiting T-cell proliferation and function. Elevated circulating and tumor-infiltrating MDSC levels are associated with poor prognosis and therapy resistance in many cancers [51].

Endothelial cells and pericytes contribute to the formation of abnormal, leaky tumor vasculature that sustains rapid tumor growth but also generates hypoxic regions. Markers of angiogenesis, such as vascular endothelial growth factors (VEGF), CD31, and CD34, provide valuable insights into tumor vascularization and represent prognostic biomarkers and therapeutic targets for anti-angiogenic therapies [52–60]. Complementing these cellular elements, the non-cellular components of the TME play an equally critical role in modulating tumor behavior and generating biomarker complexity. The extracellular matrix (ECM) provides structural support and functions as a biochemical and mechanical regulator of tumor cell behavior. ECM remodeling, mediated by CAFs and MMPs, alters tissue stiffness and composition, influencing cell migration, invasion, and drug delivery [61, 62].

Elevated collagen deposition, increased hyaluronan content, and altered ECM stiffness have all been proposed as potential biomarkers reflecting tumor aggressiveness and therapeutic resistance. Soluble factors, including cytokines, chemokines, and growth factors, facilitate communication between tumor cells and stromal elements [63]. For example, IL-6, IL-8, CCL2, and TGF-β promote tumor proliferation, angiogenesis, and immune suppression and serve as circulating biomarkers that can be measured non-invasively [64].

TME is often characterized by hypoxia and acidosis, which drive metabolic adaptation, angiogenesis, and immune escape. Hypoxia-inducible factors (HIFs) and lactate dehydrogenase (LDH) levels are among the biomarkers reflecting these physiological stressors [65]. Central to the functional complexity of the TME is intercellular communication, which orchestrates tumor progression and metastasis. Tumor and stromal cells communicate through direct cell–cell contact, soluble factors, extracellular vesicles, and immune checkpoint signaling. Soluble mediators, such as VEGF, TGF-β, and chemokines recruit endothelial cells, activate fibroblasts, and regulate immune cell infiltration, while extracellular vesicles (EVs), including exosomes and microvesicles, carry DNA, RNA, proteins, and lipids that can reprogram recipient cells [66, 67].

Tumor-derived EVs have been shown to condition pre-metastatic niches in distant organs, facilitate immune evasion, and enhance metastatic potential. EV-associated microRNAs, such as miR-21, miR-210, and miR-155, are being investigated as liquid biopsy biomarkers, providing minimally invasive insights into tumor behavior and TME dynamics [68]. Immune checkpoint molecules, such as PD-L1, expressed on both tumor and stromal cells, further exemplify communication networks that suppress cytotoxic immune responses. In contrast, metabolic cross-talk, including lactate exchange between glycolytic tumor cells and stromal macrophages, promotes tumor survival under nutrient and oxygen stress (Figure 1) [69].

EXTRACELLULAR VESICLES: BIOLOGY AND MOLECULAR CARGO

Extracellular vesicles (EVs) are membrane-bound vesicles secreted by virtually all cell types that function as critical mediators of intercellular communication, reflecting the physiological or pathological state of their cells of origin and serving as rich reservoirs of potential biomarkers in cancer

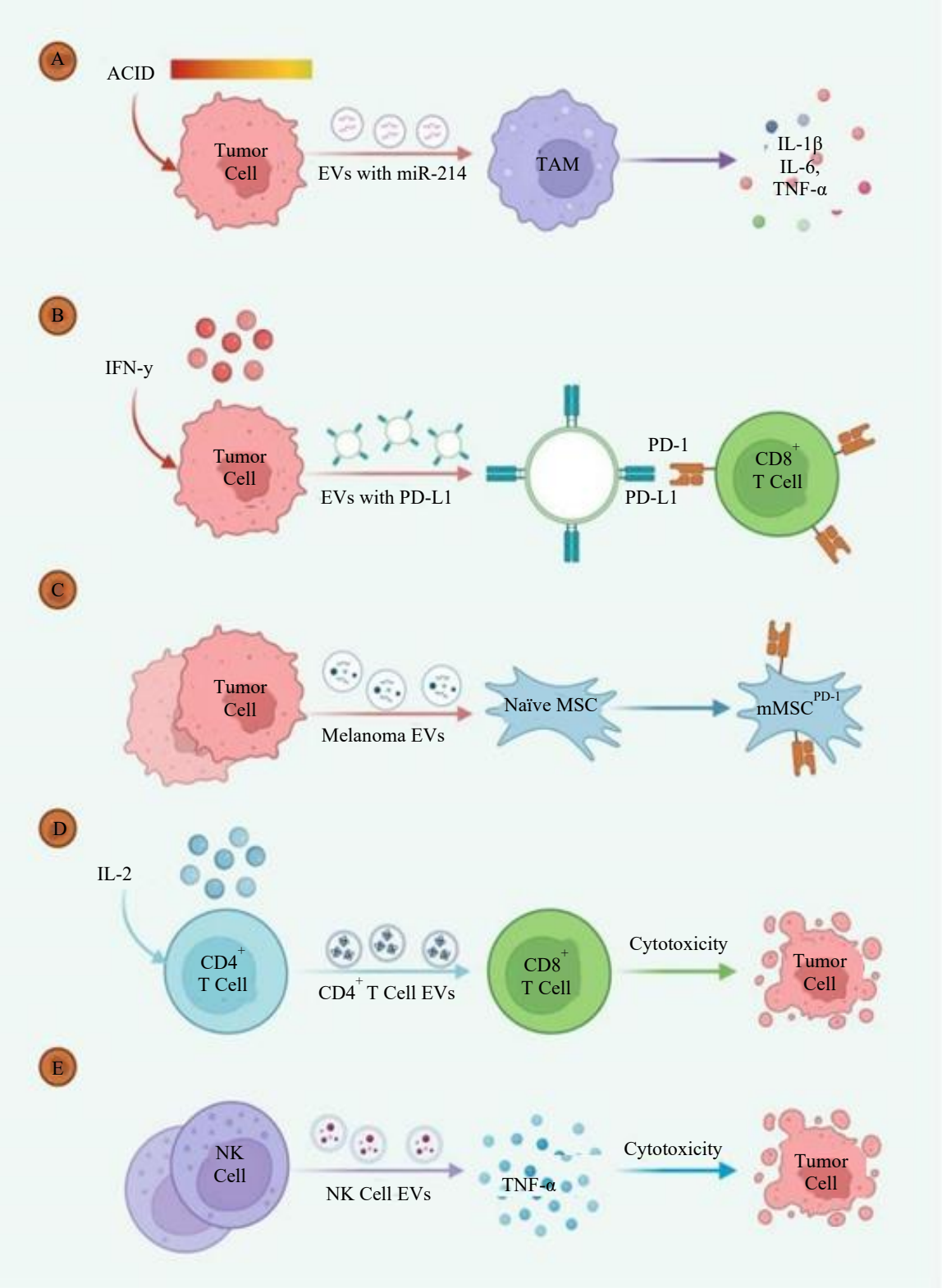

Figure 1. Extracellular vesicles mediate tumor–immune cell communication, promoting immune suppression or cytotoxic anti-tumor responses [1].

and other diseases [70]. They have gained immense attention over the past decade due to their role in modulating tumor growth, metastasis, immune evasion, and therapy resistance [71]. EVs are classified into three main categories based on their size, origin, and biogenesis pathways: exosomes,

microvesicles, and apoptotic bodies [72]. Exosomes are the smallest, typically ranging from 30 to 150 nm, and are generated through the endosomal pathway, wherein inward budding of the endosomal membrane forms intraluminal vesicles within multivesicular bodies, which are subsequently secreted into the extracellular milieu [73]. Exosomes are enriched in tetraspanins, such as CD9, CD63, and CD81, ESCRT complex proteins, heat shock proteins, and various signaling molecules, making them particularly informative of the parent cell's functional state. Macrovesicles, also referred to as ectosomes, are larger (100–1,000 nm) and arise via direct outward budding from the plasma membrane, a process regulated by cytoskeletal remodeling, phospholipid redistribution, and activation of membrane-associated enzymes, encapsulating membrane proteins, cytosolic enzymes, and nucleic acids [74, 75].

Apoptotic bodies, the largest EVs (500–2,000 nm), are released during programmed cell death and contain fragmented nuclear material, cytoplasmic components, and organelles; while traditionally associated with cellular debris clearance, apoptotic bodies also contribute to intercellular signaling, particularly in modulating immune responses and the tumor microenvironment (TME). The distinctions between these EV subtypes are structural and functional, influencing their molecular cargo, uptake by recipient cells, and the downstream signaling pathways they modulate [76]. The functional significance of EVs is intrinsically tied to their molecular cargo, which includes nucleic acids, proteins, lipids, and metabolites, enabling them to transmit complex biological information and regulate cellular behavior in local and systemic contexts. Nucleic acids within EVs comprise DNA fragments, messenger RNA (mRNA), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) [77]. Tumor-derived EVs carrying oncogenic DNA or RNA sequences can induce genetic and epigenetic changes in recipient cells, promoting tumorigenesis, epithelial-to-mesenchymal transition (EMT), and therapy resistance. Specific miRNAs, such as miR-21, miR-210, and miR-155, have been extensively studied for their role in modulating angiogenesis, immune evasion, and metastatic processes [78]. They are detectable in circulation, providing a minimally invasive source of biomarkers. Proteins encapsulated within EVs include tetraspanins, integrins, growth factor receptors, adhesion molecules, enzymes, and chaperones such as heat shock proteins. These contribute to vesicle formation, facilitate cellular uptake, and modulate signaling pathways in recipient cells [79]. EV-associated proteins have emerged as clinically relevant biomarkers, as their presence and abundance often correlate with tumor stage, progression, and response to therapy. Lipids in EV membranes, including sphingomyelin, cholesterol, phosphatidylserine, and ceramides, not only stabilize the vesicular structure but also play active roles in vesicle biogenesis, trafficking, and interaction with target cells; variations in lipid composition can reflect pathological conditions and thus serve as potential diagnostic indicators [80, 81]. Metabolites, such as amino acids, nucleotides, and small bioactive molecules within EVs, are increasingly recognized for their ability to reprogram recipient cell metabolism, support proliferation under nutrient- or oxygen-deprived conditions, and modulate the TME, highlighting another layer of functional and biomarker significance. The tumor microenvironment, EVs mediate multifaceted mechanisms of intercellular signaling that influence tumor progression, immune modulation, and metastatic dissemination [82]. Tumor-derived EVs can horizontally transfer nucleic acids to recipient cells, altering gene expression programs and promoting EMT, invasion, and metastasis [83]. EV-associated proteins, including integrins, growth factor receptors, and matrix-degrading enzymes, activate intracellular signaling cascades that enhance angiogenesis, stromal remodeling, and tumor cell survival. Immune modulation represents a critical aspect of EV function within the TME; tumor-derived EVs often carry immunosuppressive molecules, such as PD-L1, Fas ligand (FasL), and TGF-β, which inhibit cytotoxic T-cell activity, expand regulatory T-cell populations, and polarize macrophages toward pro-tumor M2 phenotypes. EV-mediated transfer of metabolic enzymes and metabolites supports adaptive metabolic reprogramming, allowing tumor and stromal cells to survive and proliferate under hypoxic and nutrient-deprived conditions common in rapidly growing tumors [84, 85].

EVs can also facilitate the formation of pre-metastatic niches in distant organs by conditioning local stromal cells and extracellular matrix components, thus preparing a favorable environment for subsequent metastatic colonization [86]. The selective packaging of specific molecules within EVs,

their targeted delivery, and the modulation of recipient cell behavior collectively underscore their role as active drivers of tumor progression and critical components of TME dynamics. The role of EVs as biomarkers stems from their reflective nature and accessibility in body fluids, making them particularly suitable for non-invasive diagnostic and prognostic applications. Circulating EVs carry tumor-specific miRNAs, mRNAs, DNA fragments, and proteins that can indicate tumor burden, stage, and therapeutic response. For example, elevated levels of miR-21-containing EVs have been correlated with aggressive disease and poor prognosis in breast, lung, and pancreatic cancers. In EV-associated PDL1 levels can predict immune checkpoint inhibitor therapy response. Nucleic acid and protein content, lipid, and metabolite profiles within EVs are being explored as diagnostic indicators, with emerging evidence suggesting that EV lipid signatures may distinguish between tumor subtypes or disease progression states. Integrating EV cargo analysis with conventional imaging, tissue biopsy, and molecular profiling can thus enhance the precision of cancer diagnosis and patient stratification. EVs also serve as mediators of therapeutic resistance, further highlighting their clinical significance. Tumor-derived EVs can transfer drug efflux transporters, anti-apoptotic proteins, and miRNAs that confer chemoresistance to recipient cells.

In contrast, EVs derived from stromal components of the TME can provide survival signals that protect tumor cells during therapy. Understanding these mechanisms provides opportunities to monitor therapy resistance via circulating EVs and potentially target EV-mediated signaling pathways to overcome resistance. EVs' biogenesis and molecular composition are tightly regulated, reflecting the state of the parent cell and the TME. Stress conditions, such as hypoxia, oxidative stress, and nutrient deprivation, can influence EV production, cargo selection, and release, thereby modulating their signaling capacity and biomarker profile. Furthermore, EV-mediated communication is highly context-dependent, with recipient cell type, local microenvironmental conditions, and receptor–ligand interactions determining the downstream biological effects. This contextual specificity contributes to EVs' functional versatility and the complexity of interpreting EV-derived biomarkers. Advances in EV isolation, characterization, and high-throughput molecular profiling have accelerated their application in cancer research and precision medicine.

Techniques, such as ultracentrifugation, size-exclusion chromatography, immunoaffinity capture, and microfluidic-based platforms, enrich EV subpopulations, while next-generation sequencing, mass spectrometry, and metabolomic approaches provide detailed cargo analysis. By combining these technologies with liquid biopsy approaches, researchers can monitor tumor dynamics in real time, detect minimal residual disease, and identify early signs of metastasis, all through the analysis of circulating EVs. The extracellular vesicles represent a multifaceted intercellular communication system integrating biogenesis, molecular cargo, and signaling within the TME. Their ability to transport nucleic acids, proteins, lipids, and metabolites allows them to modulate tumor progression, immune responses, metabolic adaptation, and metastatic niche formation, while simultaneously serving as accessible biomarkers that reflect tumor status and therapeutic response. The study of EVs thus bridges fundamental tumor biology with translational applications, offering unique opportunities for non-invasive diagnostics, prognostics, and therapeutic intervention. By unraveling the complexity of EV-mediated signaling, researchers can develop more precise biomarker-driven strategies and explore novel therapeutic avenues that target both tumor cells and the dynamic ecosystem of the TME (Figure 2).

Extracellular vesicles (EVs) have emerged as pivotal mediators of intercellular communication in cancer, functioning not only as carriers of molecular cargo but also as critical indicators of tumor behavior, disease progression, and therapeutic response, thereby establishing their potential as non-invasive biomarkers in oncology. One of the most significant roles of EVs is in immune modulation and tumor immune escape, whereby tumor-derived vesicles actively manipulate the immune microenvironment to favor tumor survival. These EVs often carry immunosuppressive molecules, such as programmed death-ligand 1 (PD-L1), Fas ligand (FasL), transforming growth factor-beta (TGF-β), and other cytokines that inhibit cytotoxic T-cell proliferation, induce apoptosis in effector T cells, expand regulatory T-cell populations, and polarize macrophages toward pro-tumor M2 phenotypes.

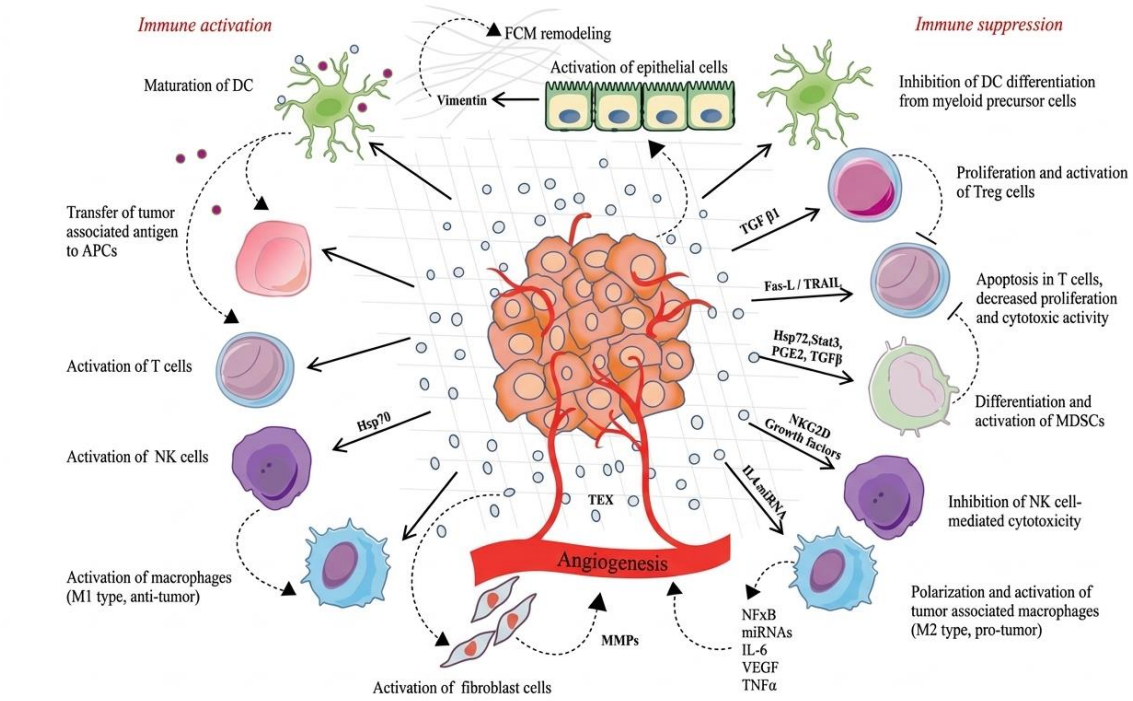

Figure 2. Ev-derived biomarkers in oncology [2].

Such immune-modulatory cargo enables tumors to evade immune surveillance, contributing to disease progression and resistance to immunotherapy. Circulating levels of PD-L1-bearing EVs and other immunosuppressive vesicles have been correlated with poor prognosis and therapy resistance in multiple cancer types, including melanoma, non-small cell lung cancer, and breast cancer, providing a minimally invasive biomarker to monitor immune status and predict response to immune checkpoint inhibitors. EV-associated microRNAs, such as miR-21, miR-155, and miR-210, have been shown to reprogram immune cells, suppress dendritic cell maturation, and promote T-cell exhaustion, highlighting the capacity of EVs to integrate multiple layers of immune regulation into systemic circulation, offering a window into tumor–immune dynamics that can be exploited for patient stratification and therapy guidance. Immune modulation, EVs play a central role in angiogenesis and metastatic dissemination, two hallmarks of cancer progression, tightly intertwined with tumor aggressiveness. Tumor-derived EVs transport a variety of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and angiogenic microRNAs, such as miR-210 and miR-132, which collectively stimulate endothelial cell proliferation, migration, and capillary formation, thereby remodeling the tumor vasculature to support rapid growth and nutrient supply (Figure 3).

EVs contribute to pre-metastatic niche formation by delivering integrins, chemokines, extracellular matrix-modifying enzymes, and other signaling molecules to distant organs, conditioning the stromal and immune microenvironment to favor metastatic colonization. For instance, specific integrin expression patterns on EV surfaces, such as integrin α6β4 or αvβ5, have been shown to determine organotropism, directing metastatic spread to the lungs, liver, or brain depending on the tumor type. These EV-mediated processes not only promote tumor dissemination but also provide measurable biomarkers; the presence of organ-targeted integrins or angiogenic factors in circulating EVs can indicate metastatic potential, disease stage, and tumor aggressiveness, enabling dynamic risk assessment and early detection of metastatic progression in cancer patients. EVs are also instrumental in therapy resistance and treatment response monitoring, representing both functional mediators of drug resistance and indicators of therapeutic efficacy. Tumor-derived EVs can encapsulate drug efflux transporters, such as P-glycoprotein, multidrug resistance-associated proteins (MRPs), and anti-apoptotic proteins, like BCL-2, which, when delivered to neighboring tumor or stromal cells, confer

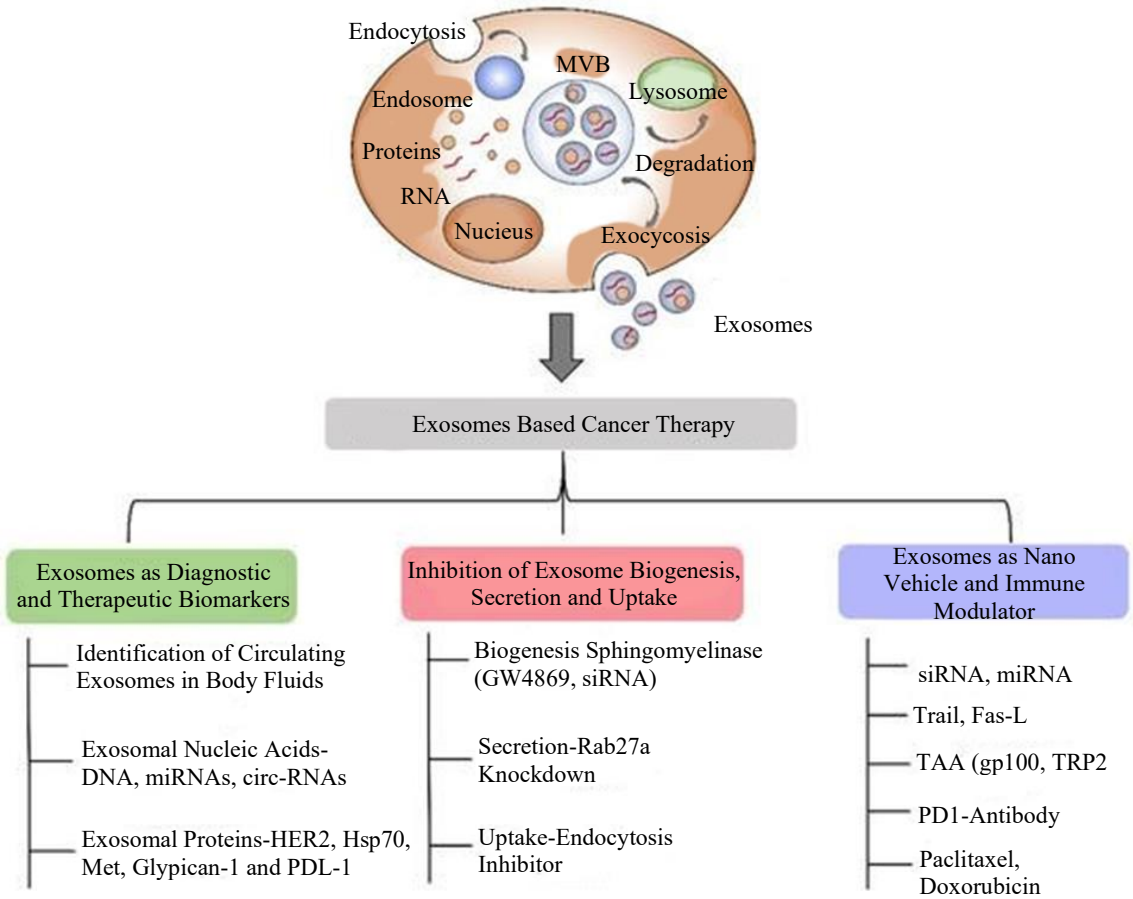

Figure 3. Exosome biogenesis and its roles in cancer diagnosis and therapy.

resistance to chemotherapy or targeted therapy. EVs carrying specific microRNAs, long non-coding RNAs, or circular RNAs can modulate gene expression in recipient cells, promoting survival pathways, inhibiting apoptosis, and altering DNA repair mechanisms, thereby contributing to multidrug resistance. EV-mediated transfer of immunosuppressive factors or checkpoint molecules can also diminish the efficacy of immunotherapies by creating a more tolerant tumor microenvironment. Clinically, longitudinal monitoring of circulating EV cargo provides a non-invasive means to evaluate treatment response in real time. Changes in the abundance of specific EV-associated proteins, RNAs, or lipids during therapy can indicate early signs of resistance, disease progression, or recurrence, offering a dynamic and personalized approach to therapeutic monitoring that complements conventional imaging and tissue biopsy. The utility of EV-derived biomarkers extends across multiple cancer types, including solid tumors and hematological malignancies, and encompasses prognostic, predictive, and pharmacodynamic applications. Elevated levels of EV-associated miR-21 and miR-1246 in breast cancer have been correlated with tumor progression and chemoresistance, whereas in lung cancer, PD-L1-positive EVs predict response to checkpoint inhibitor therapy. In colorectal and pancreatic cancers, EV integrin signatures and pro-angiogenic cargo have been linked to metastatic organotropism and patient survival. The molecular profiling of EVs with clinical parameters and imaging data allows for multi-dimensional patient stratification, facilitating precision oncology approaches that tailor treatment based on tumor biology, immune landscape, and metastatic potential. Advances in EV isolation, enrichment, and characterization technologies have greatly enhanced the feasibility of EV-based biomarkers in clinical oncology. Techniques, such as differential ultracentrifugation, size-exclusion chromatography, immunoaffinity capture, and microfluidic platforms, enable the isolation of EVs with high purity and specificity. Subsequent molecular analyzes, including next-generation sequencing, proteomics, metabolomics, and lipidomics, allow comprehensive cargo profiling, providing a detailed

snapshot of tumor-derived signals circulating in the bloodstream. These methods support longitudinal monitoring, enabling clinicians to track changes in EV composition over treatment, detect minimal residual disease, identify emerging resistance mechanisms, and predict recurrence, all with minimally invasive sampling. Integrating EV-derived biomarkers into clinical workflows can revolutionize patient management by providing real-time insights into tumor dynamics, immune evasion, vascular remodeling, and metastatic progression.

TECHNOLOGICAL ADVANCES IN EV DETECTION AND ANALYSIS

The rapidly evolving field of extracellular vesicle (EV) research has been propelled forward by significant technological advances in detection, isolation, and analysis. These have enabled the detailed study of EV biology and facilitated their translation into clinical applications such as biomarkers, therapeutic targets, and delivery vehicles. Isolation of EVs from complex biological matrices, such as blood, urine, saliva, and cerebrospinal fluid, represents a critical first step. Various techniques have been developed to enhance yield, purity, and reproducibility. Ultracentrifugation, long considered the gold standard, relies on sequential high-speed centrifugation to separate EVs based on density and size, allowing enrichment of exosomes, microvesicles, and apoptotic bodies; however, this method can be time-consuming, requires specialized equipment, and may co-isolate protein aggregates or lipoproteins. To overcome these limitations, precipitation-based approaches using polyethylene glycol or other reagents have been employed to aggregate EVs for easier collection, offering a rapid, scalable alternative suitable for clinical workflows, although these methods can compromise purity. Recent advances in microfluidics and nanotechnology-enabled isolation have revolutionized EV separation by exploiting size, density, immunoaffinity, or surface marker specificity. This allows selective capture of vesicle subpopulations from minimal sample volumes with high reproducibility. Microfluidic devices integrate channels, chambers, and surfaces coated with antibodies or ligands targeting EV surface proteins, enabling simultaneous isolation and preliminary characterization in a single platform. Nanostructured materials, such as magnetic nanoparticles, nanopillars, and nanowires, provide a high surface area for EV binding, allowing efficient enrichment and the potential for on-chip downstream analysis. These innovative approaches reduce sample processing time and labor and facilitate high-throughput applications, which are essential for clinical translation and longitudinal monitoring of disease progression. Following isolation, the characterization of EVs is critical to understanding their biological function, molecular composition, and potential as biomarkers. Nanoparticle tracking analysis (NTA) has become widely used to determine particle size distribution and concentration, providing a quantitative assessment of heterogeneous EV populations in solution. Flow cytometry, particularly high-sensitivity or nano-flow platforms, enables single-vesicle analysis. It allows simultaneous evaluation of surface markers and co-expression patterns, essential for defining EV subtypes and their cellular origins. Morphological characterization using transmission electron microscopy (TEM) or cryo-electron microscopy offers high-resolution visualization of vesicle size, shape, and membrane integrity, confirming successful isolation and revealing structural heterogeneity. Beyond physical properties, the molecular cargo of EVs can be profiled using proteomics, RNA sequencing, lipidomics, and metabolomics, providing comprehensive insight into proteins, nucleic acids, lipids, and metabolites contained within the vesicles. Proteomic analysis, often conducted via mass spectrometry, identifies surface and cytosolic proteins, including tetraspanins, integrins, signaling molecules, and enzymes, many of which have been implicated in tumor progression, immune modulation, and therapy resistance. RNA-seq allows the detection of mRNAs, microRNAs, long non-coding RNAs, and circular RNAs, revealing functional signatures that regulate gene expression in recipient cells. At the same time, lipidomic and metabolomic analyzes uncover membrane composition and small molecules that influence vesicle stability, trafficking, and metabolic signaling. Combining these techniques provides a multidimensional view of EV populations, enabling functional studies and biomarker discovery. A transformative advance in EV research has been integrating multi-omics data, which allows researchers to capture the full complexity of vesicle biology and their impact on disease processes. Multi-omics approaches combine proteomics, transcriptomics, lipidomics, and metabolomics from the same EV

populations, enabling identification of coordinated molecular networks, signaling pathways, and functional modules that may be dysregulated in cancer or other diseases. For example, integrating EV protein and RNA profiles can link specific signaling ligands with their regulatory RNA counterparts, providing mechanistic insight into tumor immune evasion, angiogenesis, or metastatic niche formation. Lipidomic and metabolomic data can further contextualize metabolic adaptations in tumor-derived EVs, revealing how vesicles contribute to nutrient exchange, hypoxia adaptation, and therapy resistance. Such integrative analyzes are increasingly being applied to patient-derived EVs, allowing correlation of molecular cargo with clinical parameters such as tumor stage, metastatic burden, therapeutic response, and overall prognosis. The combination of high-throughput isolation, sensitive characterization, and multi-omics profiling thus enables the identification of robust EV-derived biomarkers. It elucidates their mechanistic roles in disease progression, therapeutic resistance, and systemic signaling. These technological advancements have accelerated clinical translation of EV-based analyzes, providing opportunities for non-invasive diagnostics, dynamic therapy monitoring, and real-time disease progression assessment. For instance, longitudinal profiling of circulating EVs can detect early signs of relapse or treatment resistance before conventional imaging reveals tumor recurrence. EV cargo, including immunosuppressive proteins, angiogenic factors, and oncogenic RNAs, can inform treatment decisions such as patient eligibility for immune checkpoint inhibitors, anti-angiogenic or targeted therapies. Microfluidic and nanotechnology platforms enable rapid isolation and on-chip molecular analysis, supporting point-of-care testing and integration into clinical workflows. Moreover, multi-omics data can refine biomarker panels, increasing specificity and sensitivity by capturing complementary layers of information, such as combining RNA signatures with protein markers, to differentiate tumor subtypes or predict metastatic potential. Emerging machine learning and computational frameworks can further analyze complex EV datasets, uncovering patterns that may predict patient outcomes or response to therapy, thereby enhancing the utility of EVs in precision oncology.

CLINICAL APPLICATIONS OF EV-BASED LIQUID BIOPSY

Extracellular vesicle (EV)-based liquid biopsy represents a transformative advancement in oncology, offering a minimally invasive approach to cancer detection, monitoring, and therapy guidance by exploiting the molecular information encapsulated within circulating EVs. In early cancer detection and minimal residual disease (MRD), EVs provide a sensitive and dynamic window into tumor biology that often precedes conventional imaging or tissue biopsy detection. Tumor-derived EVs carry DNA fragments with somatic mutations, oncogenic mRNAs, tumor-specific microRNAs, and characteristic protein markers indicative of early tumorigenesis. This allows clinicians to detect malignant processes at a subclinical stage. For instance, EV-associated microRNAs, such as miR-21, miR-1246, and miR-210, have been reported to serve as early indicators in breast, lung, and pancreatic cancers, reflecting ongoing oncogenic signaling even in the absence of detectable lesions. Additionally, EVs can be utilized to monitor MRD following surgical resection, chemotherapy, or targeted therapy, enabling the detection of residual tumor cells that may lead to relapse. Changes in the abundance or molecular profile of circulating EVs post-treatment can indicate persistent disease or emerging resistance, offering a real-time method for early intervention before radiographic recurrence is apparent. This capability is particularly valuable in aggressive malignancies, such as pancreatic adenocarcinoma, hepatocellular carcinoma, and high-grade gliomas, where early detection of recurrence significantly impacts prognosis and survival. EV-based liquid biopsy also provides prognostic and predictive biomarkers informing disease progression, metastatic potential, and therapeutic response, thereby supporting personalized oncology approaches. Tumor-derived EV cargo, such as PD-L1, FasL, and TGF-β, can serve as indicators of immune suppression and therapeutic resistance, with elevated levels correlating with poor prognosis and reduced response to immunotherapy in multiple cancer types, including melanoma and non-small cell lung cancer. EV-associated integrins and angiogenic factors, such as VEGF and FGF, have been linked to metastatic propensity and disease aggressiveness, enabling risk stratification and prediction of organ-specific metastasis. Drug-resistance-associated RNAs and proteins transported via EVs can be monitored longitudinally to assess patient response to chemotherapy, targeted therapy, or

immune checkpoint inhibitors, providing early warning of therapeutic failure. By integrating these dynamic biomarkers into patient management, clinicians can tailor treatment regimens, escalate or de-escalate therapy, and optimize timing for surgical or adjuvant interventions. To molecular cargo, multi-omics profiling of EVs, including proteomic, transcriptomic, lipidomic, and metabolomic data, enhances the specificity and sensitivity of prognostic and predictive assessments, allowing comprehensive evaluation of tumor behavior and microenvironmental interactions. The translational potential of EV-based liquid biopsy is increasingly realized through ongoing clinical trials and studies aimed at standardizing methodologies, validating biomarkers, and integrating EV analyzes into routine patient care. Numerous cancer types trials evaluate the utility of EV-derived nucleic acids, proteins, and lipids as diagnostic, prognostic, or pharmacodynamic markers. For example, breast, colorectal, lung, and pancreatic cancer studies assess EV-associated microRNAs and DNA mutations as early detection tools. At the same time, other trials explore EV PD-L1 levels as predictive biomarkers for response to immune checkpoint blockade. Efforts are also focused on validating standardized isolation techniques, such as ultracentrifugation, immunoaffinity capture, and microfluidic platforms, alongside high-resolution characterization methods, including nanoparticle tracking analysis, flow cytometry, electron microscopy, and multi-omics profiling. These clinical and translational studies aim to establish reproducible protocols, define clinically relevant thresholds, and integrate EV analyzes into decision-making algorithms, ultimately facilitating precision oncology. In addition, the longitudinal monitoring of circulating EVs allows real-time assessment of tumor evolution, therapy resistance, and early recurrence, supporting adaptive treatment strategies that can be dynamically modified according to the patient's molecular and clinical profile. Emerging computational tools and machine learning algorithms further enhance the interpretation of complex EV datasets, identifying patterns predictive of outcomes, stratifying patients based on risk, and guiding individualized therapy. Their role in monitoring existing disease, EV-based liquid biopsies offer potential applications in screening high-risk populations, identifying molecular signatures of precancerous lesions, and guiding preventive interventions. For example, individuals with genetic predispositions, chronic inflammation, or viral oncogenic exposure can be monitored for early changes in EV cargo indicative of neoplastic transformation, enabling proactive surveillance and timely clinical intervention. Integrating EV-based liquid biopsy with other non-invasive diagnostics, including circulating tumor DNA, circulating tumor cells, and imaging modalities, can create a multi-parametric approach that enhances sensitivity and specificity while reducing the reliance on invasive tissue biopsies. The scalability and reproducibility of modern EV isolation and analysis platforms support their incorporation into clinical workflows, providing a practical path toward routine implementation in oncology practice. EV-based liquid biopsy represents a versatile and powerful platform that bridges molecular tumor profiling with clinical application, offering opportunities for early detection, MRD monitoring, prognostic, and predictive assessment, and therapy guidance. By capturing tumor-specific nucleic acids, proteins, lipids, and metabolites from circulation, EVs provide real-time insights into tumor dynamics, microenvironmental interactions, and therapeutic response. Ongoing clinical trials and translational studies continue to validate and refine these approaches, establishing EV-derived biomarkers as critical tools in the era of precision oncology. The combination of advanced isolation technologies, high-resolution characterization, multi-omics integration, and computational analytics positions EV-based liquid biopsy as a cornerstone of future cancer diagnostics, prognostics, and personalized therapy, with the potential to significantly improve patient outcomes, reduce invasive procedures, and enhance the timeliness and precision of clinical decision-making in modern oncology (Figure 4).

CHALLENGES AND LIMITATIONS

The significant advancements in extracellular vesicle (EV) research and the growing recognition of their potential as biomarkers, therapeutic targets, and mediators of intercellular communication have led to several challenges and limitations impeding their widespread clinical application and translational impact. A primary obstacle is the lack of technical standardization and reproducibility across EV isolation, enrichment, and analytical platforms. Conventional methods, such as ultracentrifugation, which separate vesicles based on density and size, are time-consuming, labor-intensive, and prone to co-isolation of non-vesicular contaminants, including protein aggregates, lipoproteins, and apoptotic

debris. Precipitation-based techniques, while faster and scalable, often compromise purity and yield inconsistent results depending on sample composition and operator variability. Microfluidics and nanotechnology-based platforms, which provide high specificity and enrichment efficiency, have improved reproducibility but face standardization, throughput, and cross-laboratory validation challenges. The downstream characterization of EVs, including nanoparticle tracking analysis (NTA), flow cytometry, electron microscopy, proteomics, and multi-omics integration, further compounds the reproducibility issue, as differences in instrumentation, calibration, data processing, and interpretation can lead to significant variability in reported EV size, concentration, molecular cargo, and functional readouts. Collectively, these technical discrepancies make it difficult to compare results across studies, validate potential biomarkers, or develop clinically reliable EV-based assays, emphasizing the urgent need for harmonized protocols, standard operating procedures, and quality control frameworks that can ensure consistent and reproducible outputs in both research and clinical contexts. The major challenge stems from EV populations' intrinsic heterogeneity and biological complexity. EVs are highly diverse, encompassing exosomes, microvesicles, and apoptotic bodies that differ in size, origin, biogenesis pathways, molecular content, and functional properties, even when derived from the same cell type. This heterogeneity is further amplified in pathological conditions, such as cancer, where tumor, stromal, and immune cells release EVs with distinct cargo and signaling properties, reflecting local microenvironmental cues, hypoxia, nutrient availability, and therapy-induced stress. The dynamic nature of EV composition complicates the identification of universally applicable biomarkers, as the abundance of specific nucleic acids, proteins, lipids, or metabolites can fluctuate over time, disease stage, or treatment course. EVs carry molecular signatures from multiple cellular sources, making it challenging to definitively attribute specific functional or biomarker effects to a particular cell type without highly selective isolation and characterization methods. This biological complexity also affects functional studies, as recipient cells may respond differently to EVs depending on the vesicle subtype, cargo composition, and microenvironmental context, limiting the interpretability and translatability of experimental findings. Addressing EV heterogeneity requires developing advanced analytical tools capable of single-vesicle resolution and computational and bioinformatic approaches that can deconvolute complex multi-omics datasets and discern biologically meaningful patterns amidst population variability. The technical and biological challenges, regulatory, ethical, and clinical adoption barriers pose additional obstacles to implementing EV-based diagnostics and therapeutics in routine practice. From a regulatory perspective, EV-based assays and therapeutics development must adhere to stringent standards for clinical-grade production, including consistency, purity, potency, and safety. The lack of universally accepted reference materials, standardized isolation methods, and validated analytical pipelines complicates regulatory approval and limits the scalability of EV-based interventions. Ethical considerations also arise, particularly when EVs are derived from patient tissues, bodily fluids, or stem cells, necessitating robust informed consent, privacy protection, and transparency regarding patient-derived biological materials. Integrating EV-based technologies into standard workflows requires demonstrating straightforward utility, cost-effectiveness, and clinical benefit, as well as training personnel and adaptation of laboratory infrastructure. In addition, given the complexity and dynamic nature of vesicle cargo, clinicians may face challenges in interpreting EV-derived biomarker data, necessitating the development of standardized reporting frameworks, decision-support tools, and evidence-based guidelines for clinical application. Complicating the landscape is the need for harmonized multi-center studies to validate EV biomarkers across diverse patient populations, tumor types, and disease stages. Variability in sample handling, storage conditions, and patient demographics can significantly influence EV yield, composition, and analytical outcomes, highlighting the importance of rigorous experimental design and cross-validation. Large-scale, prospective clinical trials are essential to establish the sensitivity, specificity, and predictive value of EV-derived biomarkers for early detection, minimal residual disease monitoring, prognosis, and therapy response assessment. The rapidly evolving field of EV research presents a moving target for regulatory authorities, requiring flexible but rigorous frameworks that balance innovation with patient safety and assay reliability. Technological and computational limitations remain significant. While multi-omics approaches have expanded the capacity to analyze EV cargo comprehensively, the integration of high-dimensional proteomic, transcriptomic, lipidomic, and metabolomic data presents bioinformatic challenges,

including data normalization, batch effect correction, and the identification of clinically actionable signatures amidst high biological variability. Advanced machine learning and AI-driven analytical platforms show promise in addressing these challenges. Still, their application requires robust training datasets, careful validation, and clear interpretability to be trusted in clinical decision-making.

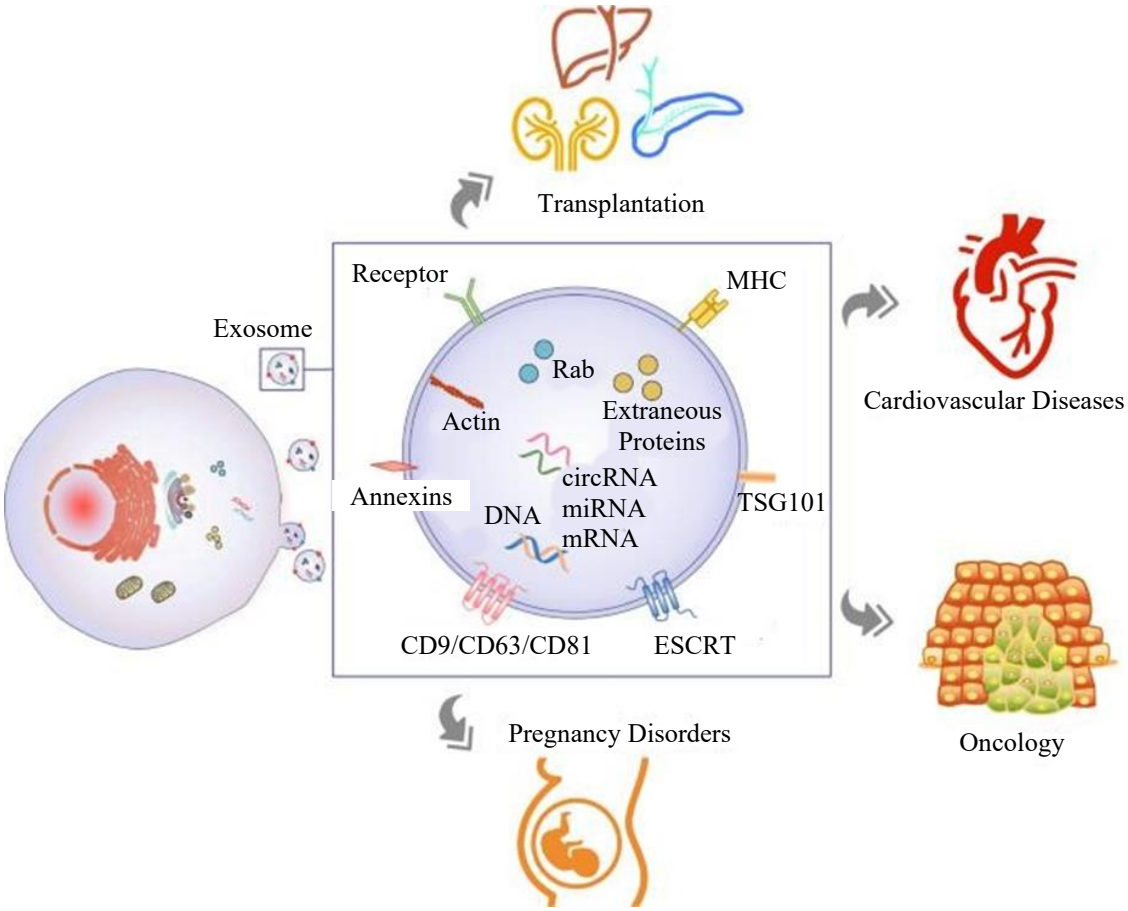

Figure 4. Exosome structure, cargo, and roles in major diseases and transplantation.

FUTURE DIRECTIONS

The landscape of extracellular vesicle (EV) research is rapidly evolving, and future directions in this field are poised to redefine cancer diagnostics, prognostics, and personalized therapy by leveraging technological innovation, multi-omics integration, and computational analytics. A central focus is integrating artificial intelligence (AI) and digital pathology with EV-based analyzes, offering unprecedented opportunities to enhance detection sensitivity, biomarker discovery, and clinical interpretation. AI-driven platforms can process high-dimensional EV datasets derived from proteomics, transcriptomics, lipidomics, and metabolomics, identifying complex patterns and signatures often indiscernible through conventional statistical approaches. Machine learning algorithms can classify heterogeneous EV populations, predict their cellular origin, and correlate specific cargo profiles with clinical outcomes such as tumor aggressiveness, metastatic potential, therapy resistance, or immune evasion. Digital pathology platforms, when combined with AI-assisted EV imaging and single-vesicle analysis, enable high-resolution visualization and automated quantification of vesicle morphology, membrane markers, and subpopulation heterogeneity. These computational approaches streamline data analysis and facilitate real-time decision-making in clinical workflows, transforming EV profiling into a practical tool for precision oncology. AI can integrate EV-derived information with other clinical parameters, imaging data, and patient demographics, generating predictive models supporting individualized therapeutic strategies, risk stratification, and longitudinal disease progression or recurrence monitoring. Multi-omics EV profiling is emerging as a cornerstone for advancing the

mechanistic understanding of tumor biology and improving biomarker robustness. By simultaneously analyzing EVs' protein, RNA, lipid, and metabolite content, researchers can capture a comprehensive molecular portrait of tumor cells, stromal components, and the tumor microenvironment. Proteomic profiling reveals key signaling proteins, tetraspanins, integrins, and enzymes that govern vesicle biogenesis, uptake, and functional impact on recipient cells. At the same time, transcriptomic analysis – including mRNA, microRNA, long non-coding RNA, and circular RNA – provides insight into gene regulatory networks, oncogenic signaling, and intercellular communication. Lipidomic and metabolomic analyzes further enhance understanding of vesicle stability, trafficking, and metabolic adaptation, uncovering how tumors modulate their environment and systemic physiology through EV-mediated metabolic reprogramming. Integration of these omics layers facilitates the identification of multi-dimensional biomarker panels that offer higher specificity and sensitivity than single analytes, enabling the detection of early-stage cancer, minimal residual disease, and emerging therapy resistance. Moreover, multi-omics EV profiling supports discovering novel therapeutic targets by elucidating functional pathways responsible for angiogenesis, immune modulation, metastasis, and drug resistance, bridging molecular insight and translational application. The prospects for routine clinical implementation of EV-based diagnostics and liquid biopsy are becoming increasingly tangible, driven by advances in standardized isolation methods, high-throughput characterization platforms, and integrative computational frameworks. Microfluidic and nanotechnology-based isolation techniques enable rapid, reproducible enrichment of specific EV subpopulations from minimal sample volumes. At the same time, high-resolution characterization approaches – including nanoparticle tracking analysis, flow cytometry, electron microscopy, and single-vesicle multi-omics – permit detailed assessment of vesicle size, morphology, and cargo composition. These advances reduce technical variability, improve assay sensitivity and specificity, and support point-of-care applications, positioning EV profiling as a feasible and practical tool in clinical oncology. Longitudinal monitoring of circulating EVs can provide real-time feedback on tumor evolution, therapy efficacy, and immune status, enabling adaptive treatment strategies and early detection of relapse or metastasis. Integrating EV-based liquid biopsy with AI-driven analytics and multi-omics profiling can transform traditional clinical workflows, replacing or complementing invasive tissue biopsies with minimally invasive, rapid, and dynamic assessments that capture tumors' molecular complexity and microenvironments.

CONCLUSION

Extracellular vesicles (EVs) have emerged as transformative mediators of intercellular communication and reservoirs of molecular information, bridging fundamental cancer biology with translational and clinical applications. Their ability to transport nucleic acids, proteins, lipids, and metabolites allows them to reflect the dynamic and heterogeneous nature of the tumor microenvironment (TME), including immune modulation, angiogenesis, metastatic dissemination, and therapy resistance. EV-based liquid biopsies provide a minimally invasive window into tumor biology, offering real-time insights into disease progression, treatment response, and minimal residual disease, while capturing spatial and temporal heterogeneity that traditional tissue biopsies often miss. Advances in isolation, characterization, and multi-omics profiling, combined with artificial intelligence and computational analytics, have greatly enhanced the sensitivity, specificity, and interpretability of EV-derived biomarkers, facilitating their integration into precision oncology frameworks. Challenges include technical standardization, EV heterogeneity, reproducibility, regulatory hurdles, and clinical adoption barriers. Addressing these limitations through harmonized methodologies, robust validation studies, and advanced analytical frameworks is essential for the reliable implementation of EV-based diagnostics and monitoring in routine clinical practice. The convergence of multi-omics EV profiling, AI-driven data interpretation, and longitudinal liquid biopsy approaches can revolutionize cancer management by enabling early detection, risk stratification, and personalized therapy. EVs represent a versatile and powerful platform that not only decodes the molecular complexity of the TME but also provides actionable insights for precision-guided cancer care, heralding a new era in oncology where minimally invasive diagnostics and real-time monitoring inform more effective, patient-specific treatment strategies.

Declarations

Ethics And Consent to Participate

Not Applicable.

Consent for Publication

Not Applicable.

Availability of Data and Materials

Not Applicable.

Competing Interests

The authors declare no competing interests.

Funding

There was no funding for this Review Article. Not Applicable.

Contributions

We all authors contributed to the conception, drafting, literature review, data interpretation, and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

The authors sincerely acknowledge the Indian Institute of Science Education and Research, Thiruvananthapuram; the Central University of Haryana, Mahendergarh; the University of Hyderabad; Indira Gandhi National Tribal University, Amarkantak; and Gujarat Biotechnology University for providing academic support and a conducive research environment. The authors also thank their mentors, colleagues, and peers for valuable discussions and insights that contributed to the preparation and refinement of this review.

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