Review

Extracellular Vesicles as Mediators of Intercellular Communication: Implications for Drug Discovery and Targeted Therapies

Md. Mahmudul Hasan * , Mst. Afsana Mimi , S. M. Sohag , Sharmin Nur Toma

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Evaluation

Extracellular Vesicles as Mediators of Intercellular Communication: Implications for Drug Discovery and Targeted Therapies

Mst. Afsana Mimi [1], S M Sohag [2], Sharmin Nur Toma [2] and Md. Mahmudul Hasan [3,*]

[1] Independent Researcher, 2-41-2 Fujigaoka, Aoba ward, Yokohama City, Kanagawa, 227-0043 Japan

[2] Graduate School of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan

[3] Earth-Life Science Institute, Institute of Science Tokyo, Tokyo 152-8550, Japan

* Correspondence: m.hasan@elsi.jp or hasan.mahmudbio@gmail.com

Abstract

Extracellular vesicles (EVs) are emerging as versatile mediators of intercellular communication and promising tools for drug discovery and targeted therapies. These lipid bilayer-bound nanovesicles facilitate the transfer of functional proteins, RNAs, lipids, and other biomolecules between cells, thereby influencing various physiological and pathological processes. This review outlines the molecular mechanisms governing EV biogenesis and cargo sorting, emphasizing the role of regulators, such as ubiquitin-like 3 (UBL3), in modulating protein packaging. We explored the critical involvement of EVs in various disease microenvironments, including cancer progression, neurodegeneration, and immune modulation. Their ability to cross biological barriers and deliver bioactive cargo renders them highly attractive for precise drug delivery systems, especially in neurological and oncological disorders. Moreover, this review highlights advances in engineering EVs for delivering RNA therapeutics, CRISPR-Cas systems, and targeted small molecules. The utility of EVs as diagnostic tools in liquid biopsies and their integration into personalized medicine and companion diagnostics were also discussed. Patient-derived EVs offer dynamic insights into disease state and enable real-time treatment stratification. Despite their potential, challenges such as scalable isolation, cargo heterogeneity, and regulatory ambiguity remain significant hurdles. We also reported novel pharmacological approaches targeting EV biogenesis, secretion, and uptake pathways, and considered UBL3 as a promising drug target for EV cargo modulation. Future directions include the standardization of EV analytics, scalable biomanufacturing, and classification of EV-based therapeutics under evolving regulatory frameworks. This review emphasizes the multifaceted roles of EVs and their transformative potential as therapeutic platforms and biomarker reservoirs in next-generation precision medicine.

Keywords: extracellular vesicles; ubiquitin-like 3; disease microenvironment; precision medicine; next-generation drug delivery

1. Introduction

Extracellular vesicles (EVs) are lipid bilayer-enclosed nanostructures secreted by almost all cell types and classified into subtypes—mainly exosomes (30–150 nm, endosomal origin), microvesicles (100–1000 nm, plasma membrane origin), and apoptotic bodies—based on their biogenesis and size. [1–3]. These vesicles carry molecular cargo such as proteins, lipids, DNA, mRNA, miRNAs, and other non-coding RNAs that influence intercellular communication [4–6]. EVs are present in nearly all biological fluids, including the blood, urine, saliva, and cerebrospinal fluid, and function as mediators of both physiological and pathological processes [2,7,8]. Under homeostatic conditions, EVs contribute to tissue regeneration, immune modulation, and neural development [9,10].

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However, in pathological settings, EVs have been implicated in cancer metastasis, neurodegeneration, cardiovascular disease, and immune dysregulation [11,12]. Their dual role as disease biomarkers and delivery vehicles highlights their relevance in diagnostics and therapeutics, particularly in precision medicine [13–15].

One of the most attractive features of EVs is their natural ability to cross biological barriers, such as the blood-brain barrier, making them suitable candidates for delivering therapeutic agents to the central nervous system (CNS) [16,17]. Their surface molecules, including integrins, tetraspanins, and other receptors, confer targeting specificity, allowing selective delivery to recipient cells and tissues [18–20].

In pharmacology and drug discovery, EVs are gaining traction as next-generation therapeutic platforms owing to their biocompatibility, low immunogenicity, and intrinsic targeting capacity [21,22]. These properties have inspired numerous preclinical and clinical investigations exploring EVs in cancer, neurodegenerative diseases, inflammation, and regenerative medicine [23–25].

In addition to their native roles, EVs are now recognized as dynamic players in the regulation of the extracellular environment and are capable of modulating host-pathogen interactions, intercellular metabolism, and even systemic physiological states. For example, microbial-derived EVs have been shown to influence tumor progression and immune responses by mimicking host-derived vesicles, revealing a complex interplay between the microbiota and host EV signaling pathways [2,26]. Furthermore, the lipid composition of EVs, especially sphingomyelins and cholesterol, contributes to their structural stability and the modulation of recipient cell signaling cascades. These lipid-rich membranes enable vesicles to fuse with target cells or interact with surface receptors, triggering downstream effects that may vary by tissue type and disease context [6,27]. Recent investigations into EV-associated enzymes and ion channels have also uncovered novel mechanisms by which EVs regulate metabolic reprogramming and stress adaptation in target cells [28]. This functional plasticity makes EVs highly attractive therapeutic platforms with adaptable properties. Importantly, leveraging omics-based analyses, such as proteomics, lipidomics, and transcriptomics, has enabled a deeper understanding of EV heterogeneity and specialization, laying the groundwork for precision-designed vesicles tailored to specific therapeutic goals [29,30].

Recent studies have demonstrated the engineering of EVs to encapsulate various therapeutic agents, including small molecules, siRNAs, miRNAs, proteins, and CRISPR/Cas9 components [31–34]. For example, exosomes engineered to express brain-targeting ligands (e.g., Lamp2b-RVG peptide) have shown enhanced central nervous system delivery via systemic administration [16]. Additionally, surface-functionalized EVs equipped with targeting peptides, antibodies, or aptamers exhibit significantly improved biodistribution and cellular uptake [35–37].

The translational potential of EVs has been demonstrated in various diseases. In oncology, EVs loaded with chemotherapeutics or siRNAs targeting oncogenes, such as KRAS, have shown antitumor efficacy [11]. In stroke and neurodegeneration, BDNF- or catalase-loaded exosomes reduce brain damage and improve functional recovery [38]. Notably, our recent study developed label-free imaging of EVs in breast cancer, highlighting their potential applications in targeted breast cancer therapies [39]. Another study conducted in 2023 reported the neuroprotective effects of EVs in neurodegenerative disease models [14]. Furthermore, neurological disorders like Parkinson's and Alzheimer's diseases have been shown to benefit from EV-based therapies, given their ability to modulate neuroinflammation and synaptic function [40,41]. A recent integrative molecular study uncovered how EVs mediate drug transport and metabolic reprogramming, further supporting their therapeutic utility [42,43].

Despite their promise, technical challenges remain, including scalable and standardized isolation, purity control, in vivo tracking, and storage stability [44,45]. The adoption of international guidelines such as MISEV2018 and MISEV2023 has provided a much-needed framework for EV characterization and nomenclature [46]. The integration of multi-omics technologies, bioinformatics, and nanotechnology is essential for overcoming the current barriers and unlocking the full clinical potential of EVs [46–48].

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2. Molecular Mechanisms of EV Biogenesis and Cargo Sorting

The biogenesis and cargo loading of EVs are controlled by coordinated molecular pathways that determine their structures and functions. EVs are broadly categorized as exosomes, formed via inward budding of late endosomes into multivesicular bodies (MVBs), and microvesicles, generated by outward budding from the plasma membrane [4,7,49].

Exosome formation is primarily governed by the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, which includes ESCRT-0 to -III and accessory proteins such as ALIX and TSG101 [50–53]. These components enable membrane budding and cargo selection. ESCRT-independent mechanisms also contribute to EV biogenesis, involving tetraspanins (CD9, CD63, and CD81), ceramides, and lipid rafts, which influence both membrane curvature and selective cargo sorting [54–56].

Rab GTPases, such as Rab27a/b, Rab11, and Rab35, regulate MVB trafficking, docking, and fusion with the plasma membrane, thereby modulating EV secretion [57–59]. For instance, Rab27a positions MVBs in the membrane, whereas Rab11 mediates the recycling pathways [60,61].

Among the emerging regulators, UBL3 (Ubiquitin-like protein 3) plays a non-canonical role in post-translational modifications and cargo sorting. UBL3 localizes to the plasma membrane and directs S-prenylation-dependent protein modifications, facilitating their selective packaging into EVs [39,62,63]. It influences the secretion of immune-regulatory and tumor-related proteins, and UBL3 deficiency disrupts EV composition in pathological contexts [39,62]. Thus, UBL3 is a promising target for the production of engineered EVs. Recent insights suggest that other post-translational modifications, such as SUMOylation and neddylation, may also contribute to selective cargo loading into EVs. These modifications influence the interaction of target proteins with sorting machinery and membrane domains, thus determining their inclusion or exclusion from vesicles [64,65]. Moreover, RNA-binding proteins, such as YBX1 and hnRNPA2B1, have been implicated in sorting miRNAs into exosomes by recognizing specific sequence motifs or structures. The phosphorylation state of these RNA-binding proteins may further modulate their activity and specificity [66]. Additionally, the lipid environment of multivesicular bodies, particularly the role of phosphatidylserine and cholesterol-rich microdomains, has emerged as a determinant of cargo affinity and membrane curvature. Together, these complex regulatory layers offer multiple intervention points for modulating EV content for therapeutic purposes, especially in diseases with aberrant intercellular communication [67,68].

The cargo content of EVs is dynamically modulated by their cellular state. Stress conditions such as hypoxia, inflammation, or oxidative stress alter EV composition, enriching them with proteins like HIF-1α, VEGF, or pro-inflammatory miRNAs [69–71]. Immune activation, for example, triggers the release of EVs carrying checkpoint proteins and miR-155, whereas neuronal activity affects EV cargo during synaptic signaling and injury responses [72,73].

Together, these tightly regulated processes ensure that EVs carry highly specific molecular signatures, enabling precise intercellular communication and presenting opportunities for the therapeutic customization of drug delivery systems. The processes of EV biogenesis and selective cargo loading are visually summarized in Figure 1 [74].

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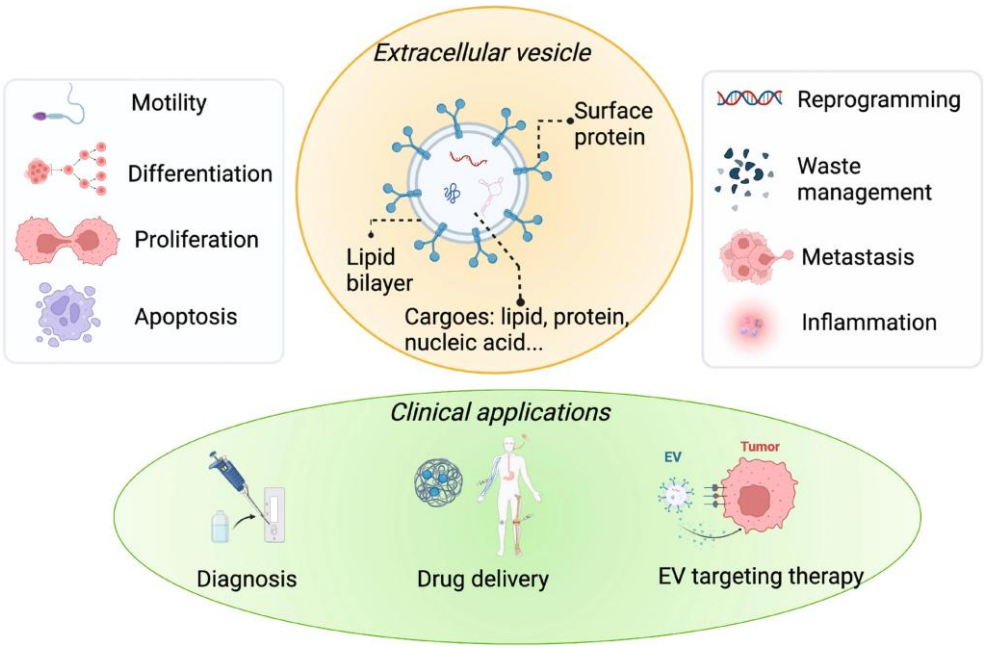

Figure 1. Molecular mechanisms of EV biogenesis and cargo sorting. EVs are generated through multiple pathways, including ESCRT-dependent and ESCRT-independent mechanisms. Rab GTPases and UBL3 also regulate EV trafficking and protein loading. Adapted and modified with permission from Anand et al., Cell Communication and Signaling (2023) [74].

3. EV-Mediated Intercellular Communication in Disease Microenvironments

EVs are critical modulators of the disease microenvironment, contributing to cancer progression, neurodegeneration, and immune modulation through the transfer of bioactive molecules, such as proteins, lipids, mRNAs, and non-coding RNAs [75–77]. Their ability to deliver specific cargo into recipient cells enables EVs to shape the behavior of neighboring or distant cells, supporting pathological processes such as tumor metastasis, inflammation, and the propagation of toxic proteins [39,62].

In the tumor microenvironment (TME), EVs mediate bidirectional communication between cancer cells and stromal, endothelial, and immune cells [78]. Tumor-derived EVs (TDEs) carry oncogenic proteins (e.g., EGFRvIII), immunosuppressive molecules (e.g., PD-L1), and pro-angiogenic factors (e.g., VEGF), promoting tumor growth, immune escape, and vascular remodeling [79–81]. TDEs also reprogram fibroblasts and recruit tumor-associated macrophages (TAMs), further amplifying their metastatic potential [82,83]. For example, exosomal integrins (α6β4 and αvβ5) have been implicated in organotropic metastasis by preconditioning distant tissues [84].

In neurodegenerative diseases, EVs act as vectors for the cell-to-cell transmission of pathogenic proteins [14,85]. Studies have shown that EVs transport misfolded tau, amyloid-β (Aβ), α-synuclein, and TDP-43, facilitating their propagation in Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [86–89]. This prion-like spread contributes to disease progression and neural network dysfunctions. Moreover, neuron- and astrocyte-derived EVs influence microglial activation, contributing to neuroinflammation during the early stages of neurodegeneration [90,91].

Extracellular vesicles (EVs) play a dual role in the immune system. On one hand, they mediate antigen presentation and T-cell activation; on the other hand, cancer-derived EVs often suppress immunity by delivering PD-L1, FasL, or miRNAs that target immune checkpoints [92,93]. Furthermore, EVs secreted by dendritic cells and T cells carry MHC-peptide complexes, costimulatory molecules, and miRNAs that modulate the activity of effector and regulatory cells, thereby influencing inflammation and tolerance [94,95]. An overview of EV functions across different disease microenvironments is shown in Figure 2 [74].

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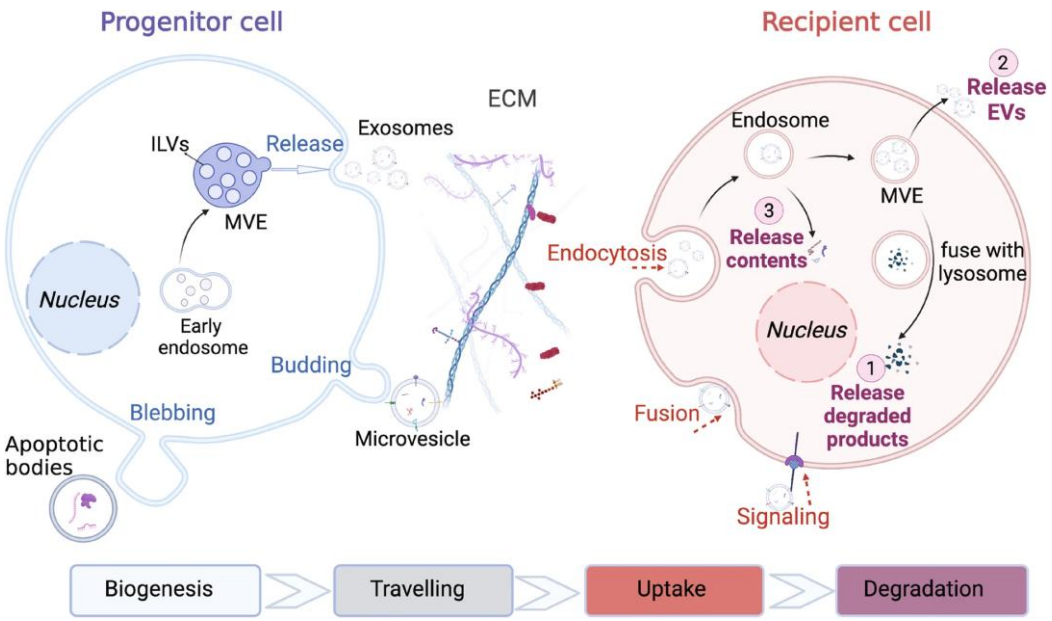

Figure 2. Schematic illustration of extracellular vesicle functions in disease microenvironments, including cancer, neurodegeneration, and immune modulation. *EVs mediate intercellular communication by transferring bioactive molecules, thereby influencing disease progression and immune response. Adapted and modified with permission from Anand et al., Cell Communication and Signaling (2023)* [74].

Emerging evidence indicates that EVs also contribute to metabolic rewiring within the disease microenvironment. Tumor-derived EVs have been shown to transfer metabolic enzymes and regulatory RNAs that alter the glycolytic or oxidative phosphorylation pathways in recipient stromal or immune cells, thereby supporting cancer cell survival under hypoxic and nutrient-deprived conditions [96,97]. In parallel, EVs released under inflammatory stress can induce metabolic shifts in macrophages, polarizing them toward a pro-tumorigenic M2 phenotype [98]. Similarly, in neurodegenerative disorders, astrocyte-derived EVs containing lactate dehydrogenase and other metabolic enzymes influence the neuronal energy balance and redox homeostasis [99]. These metabolic modulations sustain disease progression and affect therapeutic responses, especially in the context of resistance to targeted therapies. As such, EV-mediated metabolic crosstalk represents a promising yet underexplored dimension of intercellular communication that may provide novel targets for intervention in cancer and neurological diseases [100,101].

Together, EVs act as intercellular shuttles that remodel the disease microenvironment by enhancing malignancy, spreading pathogenic proteins, and modulating the immune response. Understanding these EV-mediated mechanisms opens new avenues for novel therapeutic interventions and biomarker discovery in complex diseases [102].

4. Pharmacological Targeting of EV Pathways

The pharmacological modulation of extracellular vesicle (EV) pathways is a promising strategy that can inhibit pathological EV activity and enhance therapeutic EV function. Targeting the key steps in EV biogenesis, release, uptake, and cargo packaging opens new avenues for drug development and disease intervention.

Table 1. Caption.

Agent/Strategy	Target/Mechanism	Effect on EVs	Application Context	Citation
GW4869	Inhibits neutral sphingomyelinase	Reduces exosome release	Cancer, inflammation	[103]

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Imipramine	Disrupts endolysosomal trafficking	Blocks exosome secretion	Oncology, neurodegeneration	[104]
Monensin	Alters Golgi pH, enhances EV secretion	Promotes EV release	Therapeutic EV production	[105]
Forskolin	Activates adenylyl cyclase/cAMP pathway	Increases EV release	Neuroregeneration	[106]
Dynasore	Inhibits dynamin-dependent endocytosis	Blocks EV uptake	Prevents EV-mediated signal spread	[107]
Trichostatin A	HDAC inhibitor, alters gene expression	Modifies EV cargo	Immunomodulatory EVs	[108]
Fenretinide	Inhibits dihydroceramide desaturase (DES1)	Disrupts ceramide-dependent EV release	Antitumor EV suppression	[109]
AMPK activators	Modulate cellular metabolism	Downregulate Rab27-mediated secretion	Oncogenic EV suppression	[110]
UBL3 modulation	Alters S-prenylation of surface proteins	Reprograms cargo packaging	EV engineering for precision therapy	[62,111]

Several small-molecule inhibitors that suppress EV release have been identified. GW4869, a neutral sphingomyelinase inhibitor, is widely used to block ceramide-mediated exosome biogenesis and has been shown to reduce EV-mediated inflammation and tumor progression [112,113]. Similarly, imipramine, an FDA-approved tricyclic antidepressant, inhibits exosome secretion via endolysosomal disruption [114,115]. In contrast, certain compounds such as monensin and forskolin have been reported to enhance EV release, which could be leveraged for therapeutic EV production [116,117]. A summary of the key pharmacological agents and strategies targeting EV pathways is presented in Table 1 for comparison.

The uptake of EVs by recipient cells can be pharmacologically regulated. Molecules such as dynasore and chlorpromazine inhibit clathrin- or caveolin-mediated endocytosis, thereby offering tools to prevent unwanted EV-mediated signal propagation [102,118].

Beyond trafficking, emerging methods enable the manipulation of EV content. Engineered donor cells or post-isolation techniques like electroporation, sonication, and chemical transfection allow for the loading of therapeutic cargos such as siRNAs, CRISPR-Cas systems, or chemotherapeutics [115,119].

Among the molecular targets, UBL3 has recently gained attention for its role in S-prenylation-dependent EV cargo loading. UBL3 facilitates the selective inclusion of immune and disease-related proteins into small EVs and represents a druggable pathway for cargo-level modulation [39,120]. Altering UBL3 activity may allow researchers to reprogram EV content for cancer immunotherapy or neuroinflammation modulation [39,62,121].

Together, these strategies emphasize the therapeutic potential of EV pathway modulation, positioning pharmacological EV targeting as the next-generation modality in precision medicine.

In addition to small-molecule inhibitors and engineering approaches, recent studies have explored the targeting of metabolic and epigenetic regulators that influence EV biogenesis and composition. For example, the inhibition of histone deacetylases (HDACs) with agents like trichostatin-A has been shown to modulate EV cargo by altering gene expression profiles in donor cells, enhancing the release of immunomodulatory proteins and RNAs [108,122]. Similarly, the metabolic state of a cell, governed by enzymes such as AMP-activated protein kinase (AMPK), can affect the quality and quantity of secreted EVs, which may have implications for cancer or inflammation-associated EV signaling. Pharmacological activation of AMPK has been found to

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downregulate Rab27-dependent EV secretion pathways, representing a novel method to suppress oncogenic EV spread [123,124].

Furthermore, the sphingolipid biosynthesis pathway has garnered attention as a druggable axis for EV modulation. Inhibitors of dihydroceramide desaturase (DES1), such as fenretinide, have demonstrated the ability to disrupt exosome release and reduce tumorigenic EV signaling [125,126]. These findings support a broader strategy that integrates metabolic reprogramming with EV-targeted therapy. Additionally, emerging nanoparticle-drug conjugates are being designed to bind EV surface markers (e.g., CD63, CD81), enabling selective neutralization or uptake blockade in vivo [127,128].

Collectively, these pharmacological interventions, including lipid metabolism, epigenetic regulation, and surface targeting, provide a multifaceted approach for manipulating the EV pathway. Such strategies may synergize with conventional treatments and offer precision-tuned therapeutic avenues for diseases where EVs play a central pathological role.

5. EVs as Drug Delivery Vehicles and Biomarker Reservoirs

EVs have emerged as powerful platforms for therapeutic delivery and diagnostic applications owing to their inherent biocompatibility, low immunogenicity, and natural targeting ability [129]. Their ability to transport a variety of bioactive molecules, including RNAs, proteins, and small-molecule drugs, makes them particularly promising for drug delivery and non-invasive biomarker discovery [130].

Engineered EVs have been extensively explored for the delivery of therapeutic RNAs (e.g., siRNA, miRNA, and mRNA), proteins, and genome-editing tools such as CRISPR/Cas9. Loading strategies include donor cell transfection, electroporation, sonication, and extrusion [131–134]. For example, Alvarez-Erviti et al. (2011) successfully delivered siRNA across the blood-brain barrier using exosomes engineered with the Lamp2b-RVG fusion peptide [16]. Similarly, MSC-derived EVs have been used to deliver anti-inflammatory miRNAs and neuroprotective factors in models of stroke, spinal cord injury, and myocardial infarction [135].

Surface modification techniques, such as ligand display, aptamer conjugation, and peptide anchoring, can further enhance targeted delivery. Ligands such as GE11 (for EGFR) or RGD (for integrins) have been conjugated to EV surfaces to improve tissue-specific accumulation [136,137]. These strategies significantly increase therapeutic efficacy while minimizing off-target effects and systemic toxicity.

In parallel, EVs are increasingly being recognized as rich reservoirs of diagnostic biomarkers, particularly in liquid biopsy platforms. Circulating EVs in the blood, urine, and CSF contain disease-specific proteins, lipids, and RNAs that reflect the physiological or pathological state of the originating cells [138–140]. In cancer, EV-derived miRNAs (e.g., miR-21, miR-1246), proteins (e.g., EpCAM, CD63), and DNA fragments have shown strong diagnostic and prognostic potential across various tumor types [141,142]. Similarly, EVs in neurodegenerative diseases carry α-synuclein, tau, or Aβ species, which can distinguish between disease stages and subtypes [143].

Together, these properties position EVs as multifunctional agents in drug delivery and clinical diagnostics. Their use in ongoing clinical trials further highlights their potential as the next-generation precision tools.

In recent years, researchers have also explored hybrid systems that combine EVs with synthetic nanoparticles or biomaterials to improve drug payload, release kinetics, and targeting specificity. For instance, EVs fused with liposomes or polymeric nanocarriers can synergize the benefits of natural and synthetic vectors, thereby enhancing delivery efficiency and immune evasion [144,145]. Additionally, bioengineered EVs expressing targeting ligands on their surface, such as antibodies or nanobodies, have shown promise in navigating complex tissue environments, including solid tumors and inflamed organs [146]. Another advancement includes the development of stimuli-responsive EVs that release their cargo under specific physiological triggers such as pH, redox state, or enzymatic activity, enabling precise spatiotemporal drug release [147].

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Parallel to their delivery potential, EVs are being profiled for longitudinal disease monitoring and early relapse detection. The integration of EV-based multi-omics (proteomics, transcriptomics, and metabolomics) is now feasible using high-throughput platforms, allowing for comprehensive biomarker panels with enhanced sensitivity and specificity [148,149]. This multiplexed profiling capability makes EVs particularly suitable for diseases with dynamic progression such as cancer, neurodegenerative disorders, and chronic inflammatory conditions. Moreover, coupling EV analysis with machine learning algorithms can stratify patients more accurately and predict therapeutic responses in real-time [150,151].

Collectively, these emerging technologies continue to expand the landscape of EV research, solidifying their role not just as passive carriers but also as intelligent, programmable platforms for integrated drug delivery and diagnostics in precision medicine.

6. EVs in Personalized Medicine and Companion Diagnostics

The paradigm shift toward personalized medicine has underscored the need for dynamic, minimally invasive biomarkers to guide real-time therapeutic decisions. Owing to their stability in circulation, molecular richness, and tissue specificity, EVs have emerged as promising tools in this area. EVs derived from patient biofluids (e.g., blood, urine, cerebrospinal fluid) reflect the molecular landscape of the parent cell, and thus offer a window into disease progression, therapeutic resistance, and treatment response [152,153].

6.1. Patient-Derived EVs in Cancer Monitoring

Tumor-derived EVs (TDEVs) carry oncogenic proteins, RNA, DNA fragments, and lipids that mirror the mutational status and signaling activity of the tumor. These vesicles have been detected in various cancer types, including lung, breast, prostate, and colorectal cancers, offering diagnostic and prognostic utility [154–156]. For instance, EGFR mutations and ALK rearrangements, which are critical for therapeutic selection in non-small cell lung cancer, have been identified in circulating EVs, enabling real-time monitoring of tumor evolution [157,158].

Importantly, EVs are less prone to degradation than circulating free nucleic acids and can be isolated longitudinally, allowing clinicians to track tumor heterogeneity and the emergence of drug resistance without repeated tissue biopsies [159,160]. This has profound implications for detecting minimal residual disease and recurrence earlier than imaging-based modalities.

6.2. EVs in Targeted Therapy Matching and Disease Profiling

EVs provide an opportunity to profile actionable mutations, RNA expression signatures, or proteomic alterations non-invasively, thereby supporting companion diagnostics. For example, exosomal PD-L1 expression has been correlated with immune evasion and treatment resistance in melanoma and lung cancer, thereby guiding the use of checkpoint inhibitors [161,162]. Similarly, exosomal KRAS mutations have been used to stratify colorectal cancer patients for anti-EGFR therapy [163,164].

As EVs can be sampled frequently, they enable real-time molecular profiling of disease states, helping personalize targeted therapies based on dynamic tumor biology. This is especially valuable in cases where traditional biopsies are infeasible due to tumor inaccessibility or patient frailty [165,166].

6.3. EVs in Precision Immunotherapy and Patient Stratification

EVs provide immunotherapeutic strategies that significantly benefit from EV-based diagnostics. EVs secreted by immune or tumor cells carry immune checkpoint molecules (e.g., PD-L1, CTLA-4), cytokines, and antigenic peptides that modulate immune responses [167,168]. Measuring these biomarkers in patient-derived EVs can help to predict immunotherapy responsiveness and stratify patients accordingly.

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Recent studies have demonstrated that T-cell–derived EVs enriched with CD28 or LAG3 can reflect T-cell exhaustion and immunotherapy resistance [169,170]. In addition, EV profiling has shown promise in distinguishing “hot” (inflamed) versus “cold” (non-inflamed) tumors, which are key determinants of checkpoint blockade efficacy [171].

Taken together, EVs offer a unique combination of molecular fidelity, sampling convenience, and clinical relevance. As platforms for companion diagnostics, they hold transformative potential in personalized therapy regimens, minimizing toxicity, and improving clinical outcomes in oncology and immunotherapy.

7. Challenges and Future Perspectives in EV-Based Drug Discovery

Despite their promise as drug carriers and diagnostic tools, extracellular vesicles (EVs) face several technical and translational challenges that must be overcome for their successful clinical implementation. These include issues with isolation and purification, cargo heterogeneity, dosing standardization, and an uncertain regulatory framework.

One of the primary challenges is the lack of standardized isolation protocols. Current methods, such as ultracentrifugation, size-exclusion chromatography, and precipitation, vary widely in efficiency and purity [172]. This inconsistency affects reproducibility and downstream functional analyses. Moreover, the heterogeneous nature of EV populations, even within the same biofluid, complicates their characterization and therapeutic efficacy [173]. New microfluidic platforms and affinity-based purification systems offer improved selectivity but are not yet scalable or cost-effective for clinical applications [174].

Another hurdle is the **regulatory and translational gap**. The lack of global consensus on EV classification, potency assays, and quality control has hindered the development of good manufacturing practice (GMP)-compliant EV therapies [174].

However, dosing strategies and biodistribution profiling pose significant challenges. Quantifying EVs remains difficult due to overlapping size ranges with other nanoparticles and variability in protein-to-vesicle ratios [177,178]. Additionally, the long-term effects of EV administration and immune clearance mechanisms are not yet fully understood, which raises safety concerns regarding repeated dosing.

Personalized EV-based therapeutics are expected to gain attention in the future. Patient-derived or engineered EVs tailored to individual genetic or proteomic profiles could revolutionize precision medicine [29,179]. In this context, UBL3 has emerged as a promising druggable regulator for EV cargo sorting. By modulating S-prenylation of surface proteins, UBL3 controls the selective inclusion of immune and disease-associated factors in small EVs [63]. Targeting UBL3 and its downstream pathways may allow for the precise reprogramming of EV content, particularly in cancer and neuroinflammatory conditions [39].

To further facilitate clinical translation, robust and harmonized analytics for EV pharmacokinetics and dynamics must be developed. Traditional pharmacokinetic metrics, such as half-life, clearance, and bioavailability, remain challenging to define for EVs because of their endogenous nature and diverse cargo profiles. Advanced imaging techniques and EV-specific labeling strategies, such as super-resolution microscopy or click chemistry-based tagging, are beginning to shed light on their in vivo behavior [180,181]. However, scalable quantitative tools are required. Another pressing challenge is the scalability of EV production. Current manufacturing platforms, including ultracentrifugation and tangential flow filtration, lack the necessary throughput and reproducibility for the clinical-grade EV therapeutics. Emerging bioreactor systems and cell-free synthesis approaches offer promise for industrial-scale EV generation but are still in their infancy [182]. Additionally, ethical and safety considerations must be addressed, particularly for engineered EVs carrying potent gene-editing tools or immune modulators. Regulatory authorities will need to classify EVs appropriately, whether as biologics, drug delivery systems, or cell therapy derivatives, to influence preclinical testing and approval routes [183]. Cross-disciplinary collaboration among bioengineers, clinicians, and regulatory bodies will be key to resolving these issues and realizing the

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full potential of EV-based therapies [184,185]. Additionally, inter-individual variability in EV profiles poses challenges for therapeutic standardization. Factors such as age, sex, comorbidities, and circadian rhythm can influence EV composition and efficacy, complicating reproducibility across patient populations [186].

In conclusion, although significant hurdles remain, continued advancements in EV engineering, standardization, and molecular targeting (including UBL3) are expected to accelerate the clinical translation of EV-based therapeutics in the near future.

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Abbreviations

The following abbreviations are used in this manuscript.

AD	Alzheimer's Disease
ALS	Amyotrophic Lateral Sclerosis
Aβ	Amyloid beta
BBB	Blood-Brain Barrier
CNS	Central Nervous System
CM	Conditioned Medium
CRC	Colorectal Cancer
DC	Dendritic Cell
DNA	Deoxyribonucleic Acid
EV	Extracellular Vesicle
FDA	Food and Drug Administration
GSC	Glioma Stem Cell
GTPase	Guanosine Triphosphatase
HNSCC	Head and Neck Squamous Cell Carcinoma
HSP	Heat Shock Protein
ILV	Intraluminal Vesicle
ISEV	International Society for Extracellular Vesicles
KO	Knockout
LAMP	Lysosomal-Associated Membrane Protein
miRNA	MicroRNA
miR	microRNA (generic notation)
MISEV	Minimal Information for Studies of Extracellular Vesicles
MSC	Mesenchymal Stem Cell
NSCLC	Non-Small Cell Lung Cancer
PD	Parkinson's Disease
PD-L1	Programmed Death-Ligand 1
RNA	Ribonucleic Acid
siRNA	Small Interfering RNA
sEV	Small Extracellular Vesicle
TME	Tumor Microenvironment

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TNBC	Triple-Negative Breast Cancer
UBL3	Ubiquitin-Like Protein 3
WT	Wild-Type

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