Review

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

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Review

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

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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, and other biomolecules between cells, thereby influencing various physiological and pathological processes. This review outlines the molecular mechanisms governing EV biogenesis and cargo sorting, including the role of ubiquitin-like 3 (UBL3) and its functional implications in disease microenvironments, such as cancer, neurodegeneration, and immune regulation. We further discuss recent advances in engineering EVs for the delivery of therapeutic RNAs, proteins, and small molecules, and their growing utility in noninvasive diagnostics. Despite their clinical promise, challenges such as EV isolation, heterogeneity, and regulatory standardization persist. Emerging strategies targeting EV pathways and molecular modulators such as UBL3 offer exciting prospects for precision medicine. This review highlights the current progress and future direction of positioning EVs at the forefront of next-generation drug delivery and biomarker research.

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]. 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].

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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].

Recent studies have demonstrated the engineering of EVs to encapsulate various therapeutic agents, including small molecules, siRNAs, miRNAs, proteins, and CRISPR/Cas9 components [26–29]. 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 [30–32].

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 [33]. Notably, our recent study developed label-free imaging of EVs in breast cancer, highlighting their potential applications in targeted breast cancer therapies [34]. 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 [35,36]. A recent integrative molecular study uncovered how EVs mediate drug transport and metabolic reprogramming, further supporting their therapeutic utility [37,38].

Despite their promise, technical challenges remain, including scalable and standardized isolation, purity control, in vivo tracking, and storage stability [39,40]. The adoption of international guidelines such as MISEV2018 and MISEV2023 has provided a much-needed framework for EV characterization and nomenclature [41]. The integration of multi-omics technologies, bioinformatics, and nanotechnology is essential for overcoming the current barriers and unlocking the full clinical potential of EVs [41–43].

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 [4,7,44].

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 [45–48]. 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 [49–51].

Rab GTPases, such as Rab27a/b, Rab11, and Rab35, regulate MVB trafficking, docking, and fusion with the plasma membrane, thereby modulating EV secretion [52–54]. For instance, Rab27a positions MVBs at the membrane, whereas Rab11 mediates the recycling pathways [55,56].

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 [34,57,58]. It influences the secretion of immune-regulatory and tumor-related proteins, and UBL3 deficiency disrupts EV composition in pathological contexts [34,57]. Thus, UBL3 is a promising target for the production of engineered EV.

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 [59–61]. Immune activation, for example, triggers the

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release of EVs carrying checkpoint proteins and miR-155, whereas neuronal activity affects EV cargo during synaptic signaling and injury responses [62,63].

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 process of EV biogenesis and selective cargo loading is visually summarized in **Figure 1** [64].

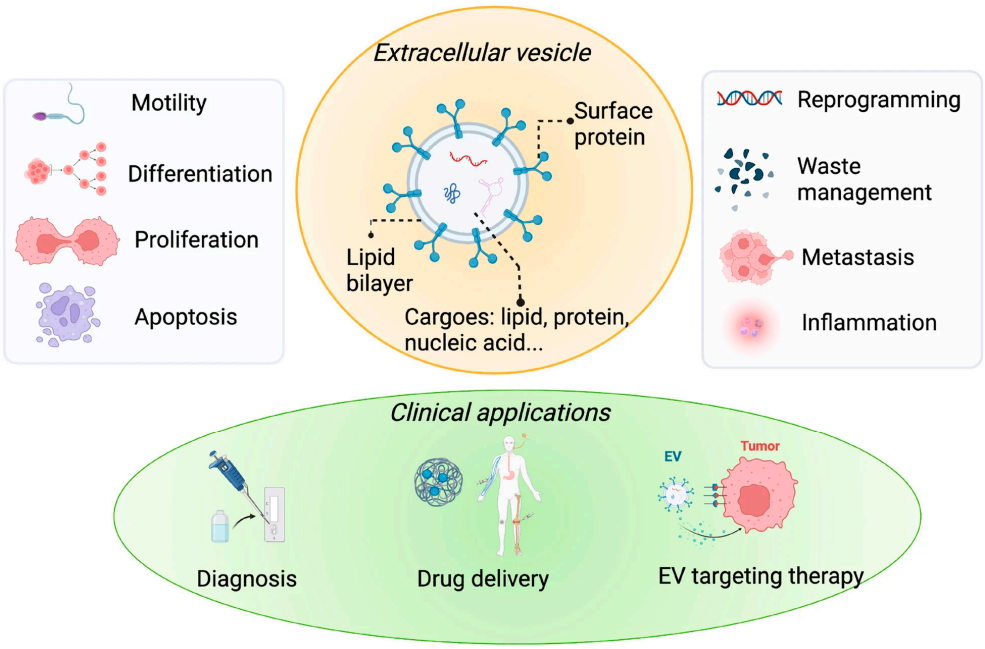

Figure 1. Molecular mechanisms of EV biogenesis and cargo sorting. EVs are generated through multiple pathways, including ESCRT-dependent and ESCRT-independent mechanisms. Rab GTPases and UBL3 also regulate EV trafficking and protein loading. Adapted and modified with permission from Anand et al., Cell Communication and Signaling (2023) [64].

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 [65–67]. 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 [34,57].

In the tumor microenvironment (TME), EVs mediate bidirectional communication between cancer cells and stromal, endothelial, and immune cells [68]. 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 [69–71]. TDEs also reprogram fibroblasts and recruit tumor-associated macrophages (TAMs), further amplifying their metastatic potential [72,73]. For example, exosomal integrins (α6β4 and αvβ5) have been implicated in organotropic metastasis by preconditioning distant tissues [74].

In neurodegenerative diseases, EVs act as vectors for cell-to-cell transmission of pathogenic proteins [14,75]. 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) [76–79]. 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 [80,81].

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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 [82,83]. 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 [84,85]. An overview of EV functions across different disease microenvironments is shown in **Figure 2** [64].

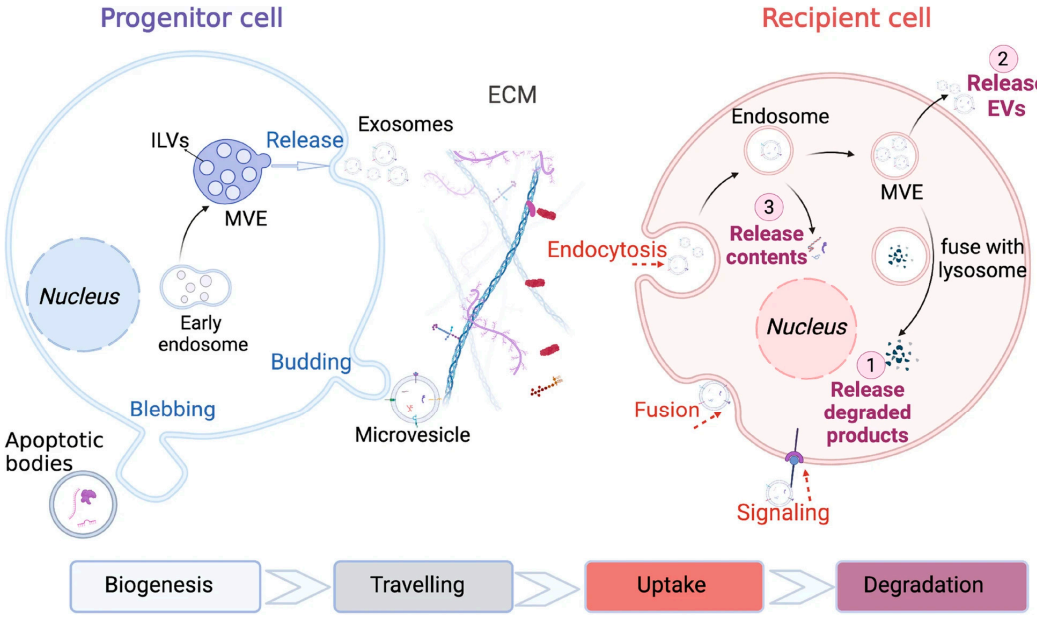

Figure 2. Schematic illustration of extracellular vesicle functions in disease microenvironments, including cancer, neurodegeneration, and immune modulation. *EVs mediate intercellular communication by transferring bioactive molecules, influencing disease progression and immune responses. Adapted and modified with permission from Anand et al., Cell Communication and Signaling (2023)* [64].

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 [86].

4. Pharmacological Targeting of EV Pathways

Pharmacological modulation of extracellular vesicle (EV) pathways is a promising strategy for both inhibiting pathological EV activity and enhancing therapeutic EV function. Targeting the key steps in EV biogenesis, release, uptake, and cargo packaging opens new avenues for drug development and disease intervention.

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 [87,88]. Similarly, imipramine, an FDA-approved tricyclic antidepressant, inhibits exosome secretion via endolysosomal disruption [89,90]. In contrast, certain compounds such as monensin and forskolin have been reported to enhance EV release, which could be leveraged for therapeutic EV production [91,92].

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 [86,93].

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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 [90,94].

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 [34,95]. Altering UBL3 activity may allow researchers to reprogram EV content for cancer immunotherapy or neuroinflammation modulation [34,57,96].

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

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 [97]. Their ability to transport a variety of bioactive molecules—including RNAs, proteins, and small-molecule drugs—makes them particularly promising for precision drug delivery and noninvasive biomarker discovery [98].

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 [99–102]. 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 [103].

Surface modification techniques, such as ligand display, aptamer conjugation, and peptide anchoring, can further enhance targeted delivery. Ligands like GE11 (for EGFR) or RGD (for integrins) have been conjugated to EV surfaces to improve tissue-specific accumulation [104,105]. 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, or CSF contain disease-specific proteins, lipids, and RNAs reflective of the physiological or pathological state of the originating cells [106–108]. 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 [109,110]. Similarly, EVs in neurodegenerative diseases carry α-synuclein, tau, or Aβ species that can distinguish between disease stages and subtypes [111].

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.

6. 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 [112]. This inconsistency affects the reproducibility and downstream functional analyses. Moreover, the heterogeneous nature of EV populations—even within the same biofluid

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complicates characterization and therapeutic efficacy [113]. New microfluidic platforms and affinity-based purification systems offer improved selectivity but are not yet scalable or cost-effective for clinical applications [114].

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 therapeutics [115,116]. The field is currently guided by position papers such as MISEV2022, but clear regulatory frameworks from agencies like the FDA or EMA remain in development [114].

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 [117,118]. 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 [119,120]. 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 [58]. Targeting UBL3 and its downstream pathways may allow for the precise reprogramming of EV content, particularly in cancer and neuroinflammatory conditions [34].

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

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

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