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Extracellular Vesicles and Circulating Nucleic Acids

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Extracellular vesicle surface proteins in tissue recognition, organotropism, biomarkers, and therapeutic engineering

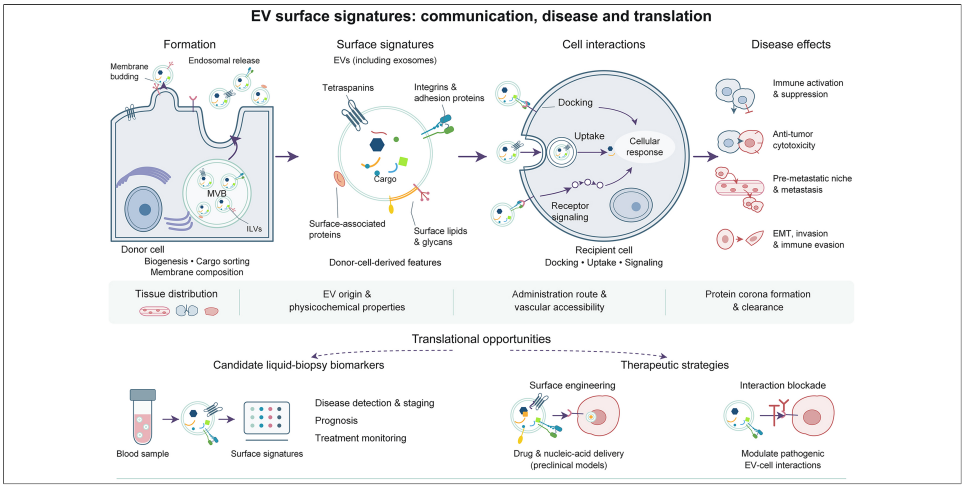
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Abstract

Extracellular vesicles (EVs), including exosomes, carry donor-cell-derived molecular features that influence their interactions with recipient cells. This review links EV biogenesis and the assembly of surface recognition cues to cellular interactions, tissue distribution, and translational applications, with an emphasis on the evidence required to interpret surface localization, biomarker value, and therapeutic outcomes. These cues can contribute to cellular docking, uptake, and receptor signaling and, together with vesicle origin, physicochemical properties, administration route, vascular accessibility, protein corona formation, and clearance, help determine tissue distribution in disease. We discuss how EV-mediated communication participates in immune activation and suppression, anti-tumor cytotoxicity, pre-metastatic niche formation, metastatic progression, epithelial-mesenchymal transition, invasion, and immune evasion. We then summarize the translational potential of EV-associated surface signatures as candidate liquid-biopsy

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biomarkers for disease detection, staging, prognosis, and treatment monitoring. Surface engineering has been explored in preclinical models, where tissue accumulation, cellular uptake, functional cargo delivery, and therapeutic efficacy are distinct outcomes; modulation of pathogenic EV-cell interactions offers another potential therapeutic avenue. Progress toward clinical application will require standardized isolation and characterization, resolution of EV heterogeneity at the single-vesicle level, reproducible biodistribution and pharmacology, scalable manufacturing, and rigorous evaluation of safety and efficacy. Defining how surface-associated features operate within this multifactorial system may clarify disease mechanisms and guide the development of more precise EV-based diagnostics and therapies.

INTRODUCTION

Extracellular vesicles (EVs) carry proteins, lipids, and nucleic acids between cells and participate in immune regulation, tissue repair, and disease progression[1,2]. Their presence in biofluids also provides a means of examining molecular features of the cells that release them. EVs comprise heterogeneous populations whose composition and functions vary with cellular origin and biogenesis. Throughout this review, EV is used as the general term, whereas "exosome" refers to vesicles with evidence supporting an endosomal origin. The term "small EV" is used when vesicle size has been characterized but the biogenesis pathway has not been established[3].

Surface proteins help determine how EVs interact with their surroundings. Integrins, tetraspanins, adhesion molecules, and receptors can participate in vesicle binding, internalization, or signaling at the recipient-cell membrane[4-7]. Their incorporation into EVs reflects both donor-cell characteristics and the pathways responsible for cargo sorting and membrane budding[8-10]. In tumor models, particular exosomal integrins have been linked to preferential uptake by lung or liver cells and subsequent changes in the local microenvironment[11]. Such findings connect vesicle composition with tissue-associated biological effects. *In vivo* distribution, however, also depends on administration route, vascular accessibility, physicochemical properties, and clearance[12,13]. The contribution of a surface protein therefore needs to be interpreted within the experimental and tissue context in which it is studied.

These interactions have implications for diagnosis and treatment, but their interpretation requires care. Detection of a protein in an EV preparation does not establish that it is exposed on the external vesicle surface or accessible to a recipient-cell receptor[14]. Likewise, increased tissue accumulation or cellular uptake does not, by itself, demonstrate functional cargo delivery[3]. Similar distinctions apply to biomarker studies: a disease-associated change in EV protein abundance provides a starting point for investigation, while diagnostic use requires reproducible measurement and validation in appropriate patient populations. Surface engineering draws on the same recognition mechanisms to improve delivery or modify immune interactions, making the relationship between surface presentation and biological activity relevant to both native and engineered vesicles[15,16].

Previous reviews have examined EV homing and tropism[17,18], as well as the composition, functions, and applications of exosomal membrane proteins[19]. Here, we examine how biogenesis and cellular origin shape EV-associated protein patterns, how surface-exposed molecules contribute to recipient-cell interactions, and how these processes relate to disease-associated functions. We use the surface "global positioning system" (GPS) concept to organize recognition mechanisms within the broader determinants of EV distribution. We then consider the evidence supporting EV-associated proteins as candidate biomarkers and the use of surface engineering for therapeutic applications. Across cancer, immune-related disorders, and other diseases, the emphasis is on connecting proposed mechanisms with the experimental findings needed to support them.

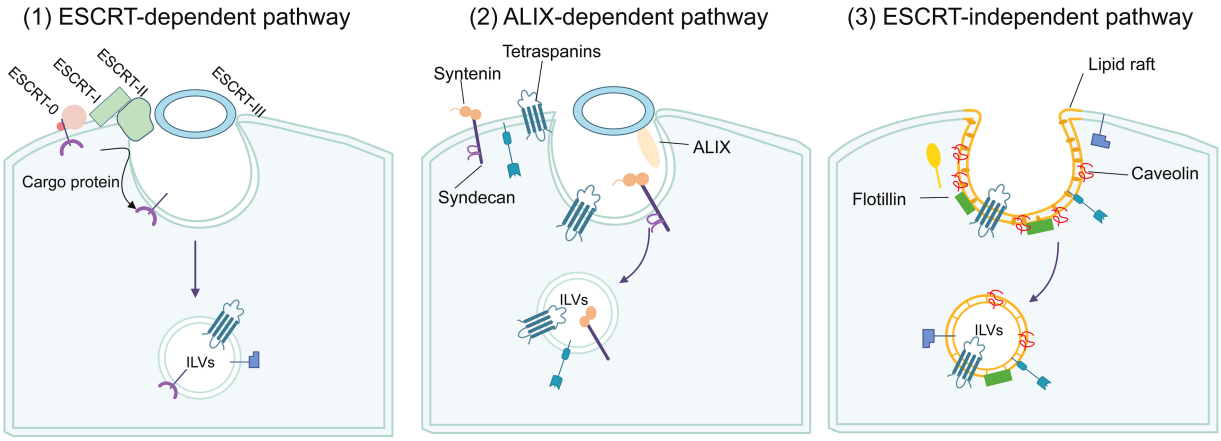


Figure 1. Pathways of intraluminal vesicle formation within multivesicular bodies: (1) canonical ESCRT-dependent; (2) non-canonical ESCRT-dependent (ALIX-dependent); and (3) ESCRT-independent pathways. Purple arrows indicate progression from membrane invagination to ILV formation, and the black curved arrow indicates cargo recruitment. Thin black lines connect labels to the indicated components; colored symbols schematically distinguish the labeled components and cargo. ESCRT-0, -I, -II, and -III denote the corresponding ESCRT complexes. ALIX: Apoptosis-linked gene 2-interacting protein X; ESCRT: endosomal sorting complexes required for transport; ILVs: intraluminal vesicles.

EXOSOME BIOGENESIS

Exosome biogenesis is an endosomal process driven by the host cell that begins when the donor cell's plasma membrane buds inward to form early endosomes[1,20], which then mature into late endosomes or multivesicular bodies (MVBs) as the endosomal membrane buds inward again to create cargo-rich intraluminal vesicles (ILVs) within the endosomal lumen[21,22]. During the initial stages of early exosome formation, membrane budding can proceed via endocytosis-dependent or endocytosis-independent pathways, ultimately forming these ILVs through three primary molecular pathways, namely, the canonical endosomal sorting complexes required for transport (ESCRT)-dependent pathway, the non-canonical ESCRT-dependent pathway, and the ESCRT-independent pathway. These distinct mechanisms shape the final surface protein composition of the resulting exosomes [Figure 1][2,23]. The exosome biogenesis pathway, summarized in Figure 2, provides a regulatory basis for shaping vesicle composition and function[24]. By influencing the surface protein repertoire, distinct assembly mechanisms can affect subsequent recipient-cell recognition, signaling, and disease-associated activities.

EV-ASSOCIATED PROTEIN PATTERNS ARE SHAPED BY DIFFERENT ILV FORMATION PATHWAYS

Interestingly, the pathways that generate ILVs within MVBs can determine which proteins are displayed on exosome surfaces by controlling cargo selection and membrane budding. In other words, the canonical ESCRT-dependent, non-canonical ESCRT-dependent, and ESCRT-independent pathways shape the exosomal surface proteome, and perturbing key components can alter specific surface markers[1,25-29].

Canonical ESCRT-dependent pathway

The canonical pathway utilizes a sequential cascade of complexes, ESCRT-0, -I, -II, -III, and vacuolar protein sorting-associated protein 4 (VPS4), to sort cargo and form ILVs[1]. ESCRT-0 recognizes various cargo proteins via its components, hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) and signal transducing adapter molecule 1 (STAM1)[25]. ESCRT-0 then binds to the ESCRT-I subunit, tumor susceptibility gene 101 (TSG101), to recruit ESCRT-I[30]. As the core component of ESCRT-I, TSG101 attracts ESCRT-II, which recruits charged multivesicular body protein 6 (CHMP6) and charged multivesicular body protein 4B (CHMP4B) to activate ESCRT-III[31]. Then, ESCRT-III drives ILV formation through sequential

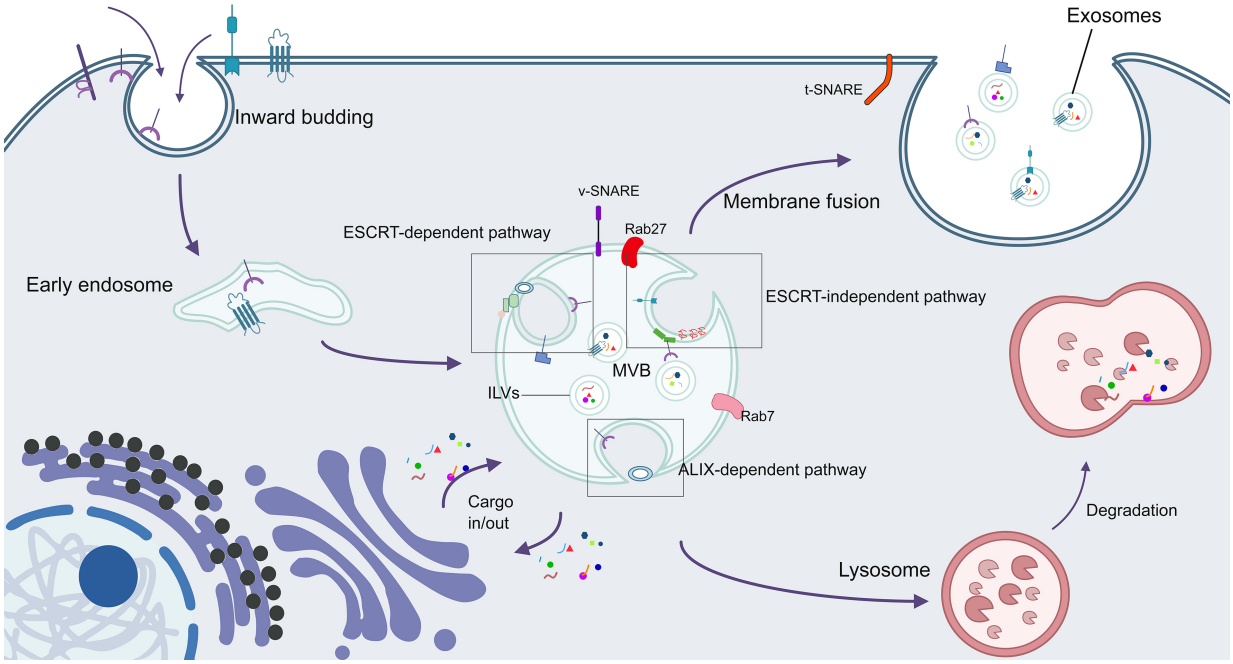


Figure 2. Overview of exosome biogenesis. Purple arrows indicate membrane internalization, endosome maturation, cargo trafficking, and the routes toward exosome release or lysosomal degradation. Thin black lines connect labels to structures, and gray boxes highlight the three ILV-formation pathways; colored symbols represent schematic membrane components and cargo. Rab27 is used as a collective label for Rab27a and Rab27b. ALIX: Apoptosis-linked gene 2-interacting protein X; ESCRT: endosomal sorting complexes required for transport; ILVs: intraluminal vesicles; MVB: multivesicular body; Rab7: Ras-related protein Rab-7; Rab27: Ras-related proteins Rab-27A and Rab-27B; SNARE: soluble N-ethylmaleimide-sensitive factor attachment protein receptor; t-SNARE: target-membrane-associated SNARE; v-SNARE: vesicle-associated SNARE.

polymerization, membrane deformation, and cleavage[32]. This cascade is able to shape exosomal surface markers[33]. Studies have shown that depleting HRS reduces secretion of cluster of differentiation 63 (CD63)- and cluster of differentiation 81 (CD81)-positive exosomes in HeLa cells[34] and decreases the exosomal surface expression of programmed death-ligand 1 (PD-L1)[35]. TSG101 actively promotes ILV budding by interacting with HRS to induce ESCRT-III recruitment[36]. In addition, TSG101 can drive reverse-topology budding by recognizing P(T/S)AP short linear motifs via its ubiquitin-conjugating enzyme E2 variant (UEV) domain[4], further influencing which proteins are incorporated into budding ILVs.

Non-canonical ESCRT-dependent pathway

The non-canonical ESCRT-dependent pathway, also known as the apoptosis-linked gene 2-interacting protein X (ALIX)-dependent pathway, bypasses ESCRT-0, -I, and -II. ALIX directly recruits ESCRT-III and VPS4[25]. Proteins in this axis are commonly detected in EV preparations[26]. ALIX serves as a central adaptor for cargo sorting by interacting with tetraspanins[cluster of differentiation 9 (CD9), CD63, CD81] and the syndecan-syntenin complex[8,37], and bridges to CHMP4B via its Bro1 domain[31]. ALIX-tetraspanin binding regulates protein cargo sorting[25] and supports incorporation of tetraspanins into vesicles during ESCRT-III recruitment[38], generating exosomes enriched in the membrane proteins CD63 and syndecan and the associated cytoplasmic adaptor syntenin. Perturbations in this pathway would remodel exosome cargo[39]. Syntenin depletion markedly reduces CD63 recovered in exosome preparations[8]. Reduced or depleted CD63 impairs exosome secretion[40] and reduces the exosomal packaging of latent membrane protein 1 (LMP1)[25,41] and vascular endothelial growth factor (VEGF)[42]. Mechanistically, ALIX drives endosomal membrane budding by binding the three LYPXnL motifs of syntenin[37], engaging ESCRT proteins such as TSG101[8], and interacting with the ESCRT-III subunit sucrose non-fermenting protein 7 (SNF7) to trigger membrane scission[43]. Notably, CD63 participates in biogenesis via ESCRT-dependent mechanisms[40] and can also be incorporated into vesicles through ESCRT-independent, ceramide-mediated budding[9], linking ALIX-dependent and lipid-driven routes.

ESCRT-independent pathway

Cells also form ILVs without ESCRT through mechanisms involving membrane-associated proteins such as caveolin-1 (CAV1) and flotillin[25], as well as glycosylphosphatidylinositol (GPI)-anchored proteins such as prion protein (PrP)[5]. This pathway comprises three steps: ILV formation, avoidance of multivesicular endosome (MVE) degradation, and MVE-plasma membrane fusion[27]. CAV1 and flotillin help sort cargo during ILV production; flotillin is essential to maintain plasma membrane architecture, cargo sorting, and exosome release[44]. Ras-related protein Rab-31 (Rab31) controls this ESCRT-independent pathway and regulates production of exosomal epidermal growth factor receptor (EGFR)[10]. Although ESCRT-dependent machinery can also sort EGFR into ILVs, those receptors are directed to lysosomal degradation rather than exosomal release[45]. Exosomal EGFR is overexpressed in several malignancies, including prostate cancer[46], implying that prostate cancer cells may preferentially utilize ESCRT-independent biogenesis. In the lipid raft-based context, flotillin can function as a membrane-associated cargo-sorting and budding regulator, providing a platform with sphingolipid ceramide and cholesterol. Here, budding is triggered by Rab31[10]. Along with ALIX and TSG101, flotillin serves as an important membrane budding regulator shaping ILV formation within MVBs[47]. Consistent with this lipid-driven pathway, CD63 can also be sorted into ILVs through ceramide-mediated, ESCRT-independent budding[9].

EV-ASSOCIATED PROTEIN PATTERNS CAN BE DETERMINED BY EXOSOME ORIGIN

Besides ILV formation pathways, the types of donor cells also greatly affect the surface proteins expressed on the exosomes[37,48,49]. Some are specific to their cell of origin, whereas others are not. More than 17% of the 271 exosomal proteins are common in the exosomes released from certain cell types such as mast cells and primary bone marrow-derived mast cells (BMMCs)[50]. These EV-associated protein profiles can reflect cellular origin and biogenesis pathways and inform subsequent biological activities[51].

Cell-of-origin-associated EV proteins

Some exosomal surface proteins are associated with their cell of origin, such as major histocompatibility complex (MHC) class I (MHC-I) and class II (MHC-II) molecules[1], cell adhesion molecules (CAMs), syndecans, transferrin receptors (TfRs), integrins, some tetraspanins[37], cluster of differentiation 3 (CD3), the glutamate receptor 2/3 (GluR2/3) subunits and A33 antigen[5]. Examples of other exosomal surface proteins that also reflect features of their donor cells are immune checkpoint proteins such as PD-L1[52] and complement regulators cluster of differentiation 55 (CD55) and cluster of differentiation 59 (CD59)[53]. MHC-II-bearing exosomes have been reported from professional antigen-presenting cells and intestinal epithelial cells[54,55]. TfRs are found in exosomes derived from reticulocytes[5]. These surface proteins can be categorized into adhesion proteins and receptor proteins[6,30,56]. MHC-I, MHC-II, CAMs, some tetraspanins such as CD81, integrins and A33 antigen belong to the adhesion type, mediating cell-to-cell or cell-to-extracellular matrix interactions. The GluR2/3 subunits of glutamate receptors and TfRs belong to the receptor type and bind to specific ligands to initiate cellular signaling pathways.

Cell-of-origin-nonspecific EV-associated proteins

Several EV-associated proteins are shared across donor-cell types, including heat shock proteins [heat shock cognate 70 (HSC70), heat shock protein 90 (HSP90)], tetraspanins (CD9, CD63, CD81), cytoskeletal proteins (actin, tubulin, moesin), ESCRT-related proteins such as TSG101, and trafficking-associated proteins such as flotillin and annexins[1,6,30]. These proteins comprise membrane proteins, membrane-associated components, intraluminal cargo, and factors involved in vesicle biogenesis. Their localization provides a basis for interpreting their roles in vesicle formation, cargo transport, and recipient-cell interactions. External surface

localization is established through experiments assessing protein accessibility on intact EVs. Surface-exposed proteins participate in interactions at the vesicle-cell interface, while other EV-associated components contribute to cargo sorting, membrane organization, and intracellular functions. CD9, CD63, and CD81 are widely used EV membrane markers. Other EV-associated proteins, including syntenin-1, epidermal growth factor receptor pathway substrate 15 homology domain-containing protein 4 (EHD4), a disintegrin and metalloproteinase 10 (ADAM10), TSG101, and annexin XI, show differential enrichment across EV populations[57,58].

IMPACTS OF EV-ASSOCIATED PROTEINS ON VESICLE BIOGENESIS

ILV formation pathways affect the exosomal surface protein pattern. In turn, EV-associated membrane proteins and biogenesis factors can affect exosome formation[59]. For example, they orchestrate selective cargo sorting, govern MVB-plasma membrane fusion and secretion, and may contribute to targeting interfaces that influence tissue tropism alongside other determinants and functional outcomes[5,8,10,21,47,60-65].

Involvement of membrane proteins and associated adaptors in selective cargo sorting

Membrane proteins and associated adaptors recruit and sort cargo via multiple, partly intersecting pathways involving syndecans, tetraspanins CD9, CD81, CD63, as well as syntenin, ALIX, and raft-associated proteins such as flotillin, stomatin, and sphingosine 1-phosphate (S1P)[10,60,66]. Among these, the syndecan-syntenin-ALIX axis is the major route. Syndecans sort syntenin into exosomal membranes through binding to syntenin's two postsynaptic density protein 95/discs large/zona occludens-1 (PDZ) domains[8,67], and syntenin-ALIX interactions then drive syndecan sorting into exosomes[8,67]. ESCRT-dependent sorting also relies on membrane-protein and adaptor interactions, with TSG101 recognizing ubiquitinated cargo through HRS and routing it into ILVs via interactions with the Bro1 domain and proline-rich region (PRR) of ALIX[4,5]. In reticulocytes, ALIX association promotes TfR sorting into exosomes, whereas HSC70 binding reduces this sorting[68].

Raft proteins contribute to cargo recruitment as well. Stomatin mediates the sorting of glucose transporter 1 (GLUT-1) into reticulocyte exosomes[60], while flotillin cooperates with Rab31 to sort EGFR and other receptor tyrosine kinases (RTKs) into ILVs. This flotillin-Rab31 axis bypasses ALIX-dependent pathways and recruits CD9, CD81, CD63, and syntenin-1 into exosomes[10]. Tetraspanins organize detergent-resistant membrane domains that recruit other membrane proteins into ILVs[5]. They sort MHC-II molecules into B-cell-derived exosomes. MHC-II has been observed in lipid-raft-associated domains of B-cell-derived exosomes[60]. Another protein, CD63, mediates the sorting of a number of proteins, including melanoma-related proteins, into ILVs in an ESCRT- and ceramide-independent manner, as well as the sorting of LMP1 into exosomes from Epstein-Barr virus (EBV)-infected cells. CD63 also binds the C-terminal fragment (CTF) of premelanosome protein (PMEL) to facilitate ILV formation and release of PMEL Mα into exosomes[15,40,41]. In parallel, a series of ligands can be recruited into exosomes independently of CD81[15]. These pathways intersect and can compensate for one another. For example, flotillin-Rab31 can bypass ALIX-dependent routes while still recruiting tetraspanins and syntenin, illustrating the overlapping nature of surface protein-driven cargo sorting into exosomes[10,60].

Involvement of membrane and biogenesis factors in MVB-plasma membrane fusion and vesicle secretion

Besides selective cargo loading, EV-associated membrane proteins and biogenesis factors also participate in MVB-plasma membrane fusion. Biogenesis begins with membrane budding within endosomes to form ILVs. As mentioned, this is a process in which ALIX, TSG101, and flotillin act as key regulators[47]. CD63 participates in vesicle formation via both ESCRT-dependent budding[40] and ESCRT-independent, ceramide-mediated mechanisms[9]. Flotillin provides structural platforms within lipid raft domains; triggered by Rab31,

flotillin coordinates sphingolipid ceramide and cholesterol to drive budding and ILV formation within MVBs[10]. As MVBs mature, they either fuse with lysosomes for degradation or with the plasma membrane for exosome secretion[61,69,70]. The trafficking and docking steps that bias MVBs toward secretion are controlled by small guanosine triphosphatases (GTPases)[71]. Studies have shown that, in HeLa cells, Ras-related protein Rab-7 (Rab7) inactivation facilitates fusion between MVBs and the plasma membrane and hence promotes exosome release[10], while Ras-related protein Rab-27B (Rab27b) transports MVBs to the cell periphery and Ras-related protein Rab-27A (Rab27a) mediates their docking at the plasma membrane[62].

Fusion at the plasma membrane is initiated when synaptotagmin interacts with syntaxin to activate the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) machinery, enabling exosome release through the SNARE complex[21,72,73]. This complex comprises Qa-, Qb-, and Qc-SNARE (glutamine-containing) and R-SNARE (arginine-containing) subfamilies[63]. Membrane proteins and associated factors further modulate these steps: the tetraspanins CD9, CD63, and CD81 contribute to membrane fusion[47], and annexins, especially annexin A2, are raft-associated factors that can recruit ILVs to the MVB limiting membrane by back-fusion, mediate docking of MVBs at the plasma membrane, and promote fusion to facilitate exosome release[5,65]. Consistent with these roles, overexpression of CD63 enhances membrane fusion and changes endosomal morphology in mammalian systems[67]. Following fusion, interaction between TSG101 and ALIX can promote abscission processes that lead to exosome release[8].

THE "GPS" CONCEPT: SURFACE-MEDIATED RECOGNITION, UPTAKE, SIGNALING, AND ORGANOTROPISM

Here, the surface "GPS" concept refers to recognition cues presented by EV-associated surface molecules that contribute to vesicle binding, cellular uptake, and receptor engagement. These interactions support cellular recognition and can contribute to organotropism, the preferential distribution or uptake of vesicles among tissues *in vivo*. Tumor-derived exosomes (TDEs) bearing integrins α6β4 and α6β1 were reported to show preferential lung uptake, whereas those enriched in αvβ5 showed uptake by liver Kupffer cells and contributed to pre-metastatic niche formation[11]. Biogenesis pathways shape the protein and lipid composition of EVs through cargo sorting and membrane assembly. The syndecan-syntenin-ALIX axis and lipid-dependent budding represent distinct mechanisms contributing to these compositional features[8-10]. Surface-exposed recognition molecules support interactions with recipient cells through receptor binding, cellular uptake, and signaling. Cell-type-specific uptake and receptor-mediated signaling describe interactions at the cellular level; organotropism describes preferential EV distribution or uptake among tissues *in vivo*. Tissue distribution reflects the combined contributions of surface recognition cues, vesicle origin, physicochemical properties, administration route, vascular accessibility, and clearance[12,74].

Surface-associated EV components mediate uptake by recipient cells

Exosome navigation to target cells can be mediated by the docking of exosomal surface proteins to cell-surface receptors, thereby facilitating exosome uptake[75]. In the human brain microvascular endothelial cell line hCMEC/D3, annexin A2 exposed on glioma-derived EVs interacts with cell-surface heparan sulfate chains to enhance EV uptake[76]. Separately, flotillin-associated membrane domains in recipient cells participate in endocytic pathways involving GPI-anchored proteins[77]. Receptor-mediated routes also operate in other cell types. T-cell-derived exosomes bearing lymphocyte function-associated antigen 1 (LFA-1) and α4β7 integrins engage intercellular adhesion molecule 1 (ICAM-1) and mucosal addressin cell adhesion molecule 1 (MAdCAM-1) on mouse brain endothelial cells to drive uptake[78], and a complex of tetraspanin-8 (TSPAN8) with integrin α4 binds cluster of differentiation 54 (CD54) and facilitates internalization by endothelial cells[4,79]. More broadly in endothelium, tetraspanins can increase exosome uptake and thereby stimulate angiogenesis and vasculogenesis[6]; CD63 can drive tyrosine-based

internalization[80]; exosomal integrins can dock the exosomes onto recipient surfaces to promote endocytosis[81]. In immune cell populations, exosomal surface proteins have additional functions. Exosomal LFA-1 interacts with ICAM-1 to mediate the uptake into antigen-presenting cells[82] by facilitating integrin-mediated endocytosis[83]. T-cell immunoglobulin and mucin domain-containing protein 4 (Tim4) on recipient phagocytic cells recognizes phosphatidylserine on EVs[84]. In hepatic stellate cells, integrins αvβ3 and α5β1 on the recipient cells contribute to exosome binding, uptake, and functional cargo transfer[85]. In the pancreas, pancreatic cells similarly internalize exosomes through the TSPAN8-integrin α4 complex on vesicles, which binds CD54 on the recipient surface to promote endocytosis[4]. In the kidney, interactions between exosomal C-X-C motif chemokine receptor 4 (CXCR4) and stromal cell-derived factor-1α direct the uptake of endothelial colony-forming cell-derived exosomes within renal tissues[86].

In the tumor models reported by Hoshino *et al.*, integrin-associated exosome uptake was linked to Src activation and S100 gene expression in recipient cells, with effects on migration and inflammation within the pre-metastatic niche[11]. Across diverse recipient cell types, EV-associated membrane components can also support lipid raft-mediated endocytosis. Heparan sulfate proteoglycans (HSPGs) on recipient cells promote exosome uptake; HSPG-dependent internalization then activates signaling through extracellular signal-regulated kinases 1 and 2 (ERK1/2) to further drive endocytosis[87,88]. These uptake mechanisms involve proteins on both sides of the EV-cell interface: EV-surface annexin A2 can interact with recipient-cell HSPGs[76], whereas recipient-cell flotillin-associated membrane domains and ERK1/2-heat shock protein 27 (HSP27) signaling contribute to endocytosis in specific experimental models[1,77].

Surface-associated EV components activate recipient-cell receptors

Besides facilitating receptor-mediated uptake, the exosomal surface proteins can influence the recipient cells through activation of specific signaling pathways after binding to the cell-surface receptors [Figure 3] without internalization[89,90]. Fibronectin displayed on exosomes binds to integrins on fibroblasts or to HSPGs on myeloma cells[91], hence activating these receptors and triggering specific intracellular signaling pathways[77]. In fibroblasts, fibronectin-integrin engagement activates focal adhesion kinase (FAK) and Src family kinase (SFK) signaling to drive invasion[92]. In myeloma cells, the fibronectin-HSPG binding activates p38 mitogen-activated protein kinase (p38) and phosphorylated extracellular signal-regulated kinase (pERK) pathways and induces Dickkopf-1 (DKK1) and matrix metalloproteinase 9 (MMP-9) expression, promoting myeloma progression[93]. In addition, integrins β1 and β2 on B-cell-derived exosomes promote adhesion to fibroblasts and elicit intracellular Ca^{2+} signaling. Interferon gamma receptor 1 (IFNGR1) present on exosome surfaces can bind free interferon gamma (IFN-γ) on recipient cells, activating the signal transducer and activator of transcription 1 (STAT1) pathway and facilitating communication between neural stem cells and their microenvironment[23].

Taken together, the engagement of recipient-cell receptors by exosomal surface proteins and the resulting cargo uptake and/or signaling activation can regulate cellular functions and hence influence disease progression[94].

PHYSIOLOGICAL AND PATHOLOGICAL SIGNIFICANCE OF EV SURFACE-MEDIATED COMMUNICATION AND ORGANOTROPIC EFFECTS IN CANCER

Integrins, tetraspanins, adhesion molecules, and receptors on exosomal surfaces form a surface GPS that shapes recipient-cell recognition and, in some models, preferential tissue uptake. In normal physiology, this targeting supports coordinated tissue repair, angiogenesis, immune modulation, and neural-glial communication because the signals can act in receptive niches. However, in pathological conditions, the same machinery can pre-condition distant microenvironments, amplify inflammation or immunosuppression, and facilitate metastatic spread. These dynamics are especially prominent in cancer.

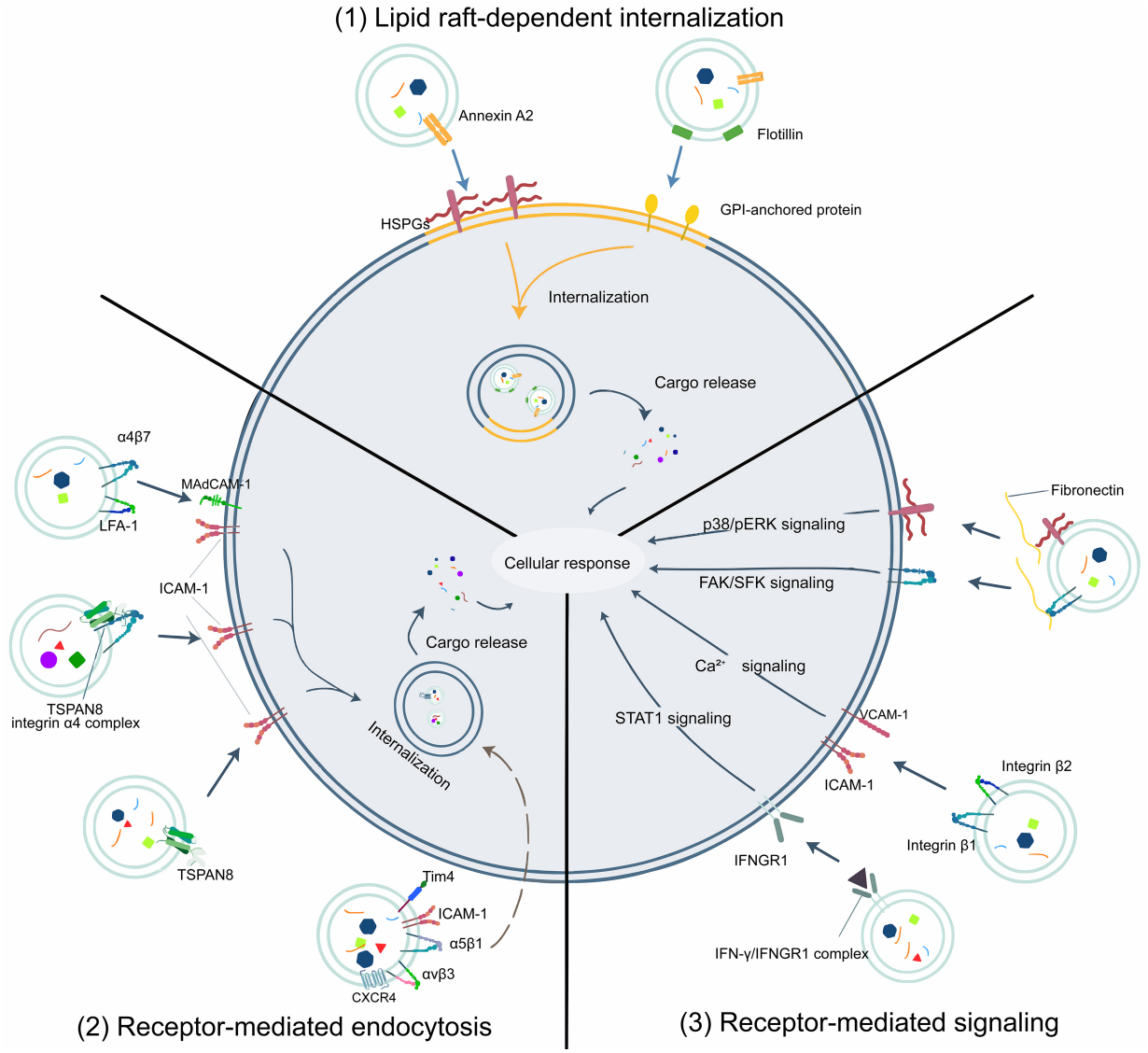


Figure 3. Mechanisms of extracellular vesicle uptake and receptor-mediated signaling in recipient cells: (1) lipid raft-dependent internalization; (2) receptor-mediated endocytosis; and (3) receptor-mediated signaling. Orange arrows indicate lipid raft-dependent internalization; blue-gray arrows indicate the illustrated EV-cell interactions, uptake, cargo release, and signaling. The dashed curved arrow indicates the illustrated uptake route toward the endosomal compartment, while thick black lines separate the three mechanisms. Integrin subunits are designated by Greek letters and numbers. Ca^{2+}: Calcium ion; CXCR4: C-X-C motif chemokine receptor 4; FAK: focal adhesion kinase; GPI: glycosylphosphatidylinositol; HSPGs: heparan sulfate proteoglycans; ICAM-1: intercellular adhesion molecule 1; IFN-γ: interferon gamma; IFNGR1: interferon gamma receptor 1; LFA-1: lymphocyte function-associated antigen 1; MAdCAM-1: mucosal addressin cell adhesion molecule 1; p38: p38 mitogen-activated protein kinase; pERK: phosphorylated extracellular signal-regulated kinase; SFK: Src family kinase; STAT1: signal transducer and activator of transcription 1; Tim4: T-cell immunoglobulin and mucin domain-containing protein 4; TSPAN8: tetraspanin-8; VCAM-1: vascular cell adhesion molecule 1.

Some exosomal surface proteins have tumor-suppressive effects [Table 1], whereas others have tumor-promoting effects [Table 2]. The TDEs can remodel extracellular matrices, instruct stromal and immune cells, and contribute to niche formation and metastatic colonization in experimental models[95].

Surface-mediated EV cytotoxicity in tissue contexts

Exosomal surface proteins can directly trigger cancer cell death in a receptor- and tissue-context-dependent manner. For example, tumor necrosis factor (TNF), Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL) on exosomes activate caspase-dependent apoptosis in cancer cells[96]. Exosomal TRAIL

Table 1. EV-associated proteins with tumor-suppressive activities

EV-associated protein(s)	Origin	Reported activity or mechanism	Cancer type	References
MHC-II, BAG6	IFN-γ-stimulated DC-derived EVs	Enhanced NKp30-dependent NK-cell function, correlated with MHC-II and BAG6 expression	NSCLC	[246]
IL-15Rα	DC-derived EVs	Immune activation (NK proliferation and IFN-γ secretion)	Multiple	[247]
NKG2D ligands	DC-derived EVs	Immune activation (direct NK activation)	Multiple	[247]
TNF	DC-derived EVs	Immune activation (stimulates NK-cell IFN-γ)	Multiple	[96]
HSP70, BAG4	Tumor-derived EVs	HSP70-positive EVs enhance NK-cell migration and cytolytic activity	Multiple	[248,249]
TNF, FasL, TRAIL	DC-derived EVs	Direct tumor apoptosis (caspase activation)	Multiple	[96]
TRAIL	TRAIL-positive EVs	TRAIL-dependent tumor-cell death; DR5-associated apoptosis in SUDHL4 and INT12	DLBCL (SUDHL4), melanoma (INT12), myeloma (KMS11)	[97]

BAG4: B-cell lymphoma 2-associated athanogene 4; BAG6: B-cell lymphoma 2-associated athanogene 6; DC: dendritic cell; DLBCL: diffuse large B-cell lymphoma; DR5: death receptor 5; EV: extracellular vesicle; FasL: Fas ligand; HSP70: heat shock protein 70; IFN-γ: interferon gamma; IL-15Rα: interleukin-15 receptor alpha; MHC-II: major histocompatibility complex class II; NK: natural killer; NKG2D: natural killer group 2 member D; NKp30: natural killer cell protein 30; NSCLC: non-small cell lung cancer; TNF: tumor necrosis factor; TRAIL: TNF-related apoptosis-inducing ligand.

induces potent apoptosis when engaging death receptor 5 (DR5) on SUDHL4 lymphoma and INT12 melanoma, resulting in 68% and 51% tumor growth inhibition, respectively[97], whereas binding to death receptor 4 (DR4) on KMS11 myeloma predominantly yields necrosis[97]. These observations indicate that TRAIL-positive exosomes can selectively suppress tumor growth depending on the distribution and signaling of death receptors in the target tissues.

Surface protein-mediated metastatic programming and organotropic effects

Exosomal integrins contribute to organotropic metastasis and microenvironmental conditioning[98]. TDEs enriched for α6β4 and α6β1 have been linked to lung metastasis, whereas αvβ5 has been implicated in uptake by Kupffer cells and hepatic niche conditioning[11,99]. Cellular αvβ6 promoted an osteolytic program in prostate-cancer models[100]. Separately, exosomal αvβ6 was transferred between prostate-cancer cells[101]. Beyond guiding tissue targeting, exosomal integrins can be transferred to neighboring tumor cells; lateral acquisition of αvβ6 increases invasiveness and accelerates metastatic outgrowth[101]. Studies showed that exosomal αvβ3 correlates with adhesion and migration in metastatic prostate cancer[102], while integrin β4 (ITGB4) in concert with EGFR and mucin-1 (MUC1) serves as a prognostic module in cholangiocarcinoma by promoting growth and dissemination[103]. Non-integrin EV-associated proteins similarly rewire tumor and stromal behavior. In metastatic hepatocellular carcinoma, exosomal caveolins, hepatocyte growth factor receptor (MET), and S100 family members convert hepatocytes toward an invasive and migratory phenotype by activating phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) signaling pathways and elevating their responsiveness to matrix metalloproteinases (MMPs)[104]. Exosomal PD-L1 and N-cadherin enhance migration of osteosarcoma cells that contribute to lung metastasis[105]. Besides, the tetraspanin-linked mechanisms are also prominent. Exosomal cluster of differentiation 44 (CD44) from ovarian cancer can be transferred to peritoneal mesothelial cells to facilitate invasion and metastasis[106]. CD44 engagement also sustains cancer stemness and activates epithelial-mesenchymal transition (EMT) to promote dissemination[107]. It has been reported that CD44 is highly expressed on doxorubicin (Dox)-resistant breast cancer exosomes, linking it to metastasis and drug resistance[108], implying that CD44 can be a therapeutic target to overcome drug resistance. Additional EV-associated mediators include cluster of differentiation 97 (CD97) and TSPAN8, which drive gastric cancer proliferation and invasion[109,110]. At the cellular level, CD63 binds tissue inhibitor of metalloproteinases 1 (TIMP-1) and supports β1 integrin signaling and survival in mammary epithelial cells[111]. Other surface

Table 2. EV-associated proteins with tumor-promoting activities

EV-associated protein(s)	EV source	Reported activity or mechanism	Cancer type	References
Integrin α6β4, α6β1	Lung-tropic breast cancer cell-derived EVs	Preferential lung uptake; lung pre-metastatic niche formation	Breast cancer	[11]
Integrin αvβ5	Liver-tropic pancreatic cancer cell-derived EVs	Preferential uptake by liver Kupffer cells; liver pre-metastatic niche formation	Pancreatic cancer	[11,99]
Integrin αvβ6	Prostate cancer cell-derived EVs	EV-mediated transfer of αvβ6 to recipient prostate cancer cells; enhanced adhesion and migration	Prostate cancer	[101]
Integrin αvβ3	Prostate cancer cell-derived EVs	Tumor progression and metastasis (adhesion/migration)	Prostate cancer	[102]
ITGB4, EGFR, MUC1	Cholangiocarcinoma-derived EVs	EV protein profile associated with cell migration, proliferation and survival pathways	Cholangiocarcinoma	[103]
CAV1, MET, S100 family	Metastatic HCC-derived EVs	Tumor progression and metastasis (reprogram hepatocytes)	Hepatocellular carcinoma	[104]
PD-L1, N-cadherin	Osteosarcoma-derived EVs	Tumor progression and metastasis	Osteosarcoma	[105]
CD44	Ovarian/breast cancer-derived EVs	Tumor progression and metastasis (invasion, drug resistance)	Ovarian/breast cancer	[106-108]
CD97, TSPAN8	Gastric cancer cell-derived EVs	Tumor progression and metastasis (proliferation/invasion)	Gastric cancer	[109,110]
PSGR	Prostate cancer cell-derived EVs	Tumor progression and metastasis; ECM remodeling and EMT (migration, stemness, EMT)	Prostate cancer	[112]
ADAM10	Cancer-associated fibroblast-derived EVs	Tumor progression and metastasis (Notch receptor and RhoA activation)	Multiple	[113]
Fibronectin 1, LAMC1	Gastric cancer-derived EVs	Tumor progression and metastasis (mesothelial adhesion, peritoneal metastasis)	Gastric cancer	[114]
B7-H3	Colorectal cancer-derived EVs	Angiogenesis; tumor progression and metastasis (AKT1/mTOR/VEGFA activation)	Colorectal cancer	[115]
PDGFR-β	Melanoma-derived EVs	Therapy resistance (MAPK inhibitor bypass); tumor progression	Melanoma	[116]
CD47	Tumor-derived EVs	Immune evasion ("don't eat me" signal)	Multiple	[19]
PD-L1	Melanoma, breast cancer and glioblastoma-derived EVs	Immune evasion (T-cell suppression)	Melanoma, breast cancer, glioblastoma	[35,123-125]
LMP1, galectin-9	Nasopharyngeal carcinoma-derived EVs	Transport of viral and immunomodulatory proteins in nasopharyngeal carcinoma EVs	Nasopharyngeal carcinoma	[250,251]
TGF-β	Tumor-derived EVs	Immune evasion (downregulate NKG2D on NK/$CD8^+$ T cells)	Multiple	[128]
TNF	Melanoma-derived EVs	ROS-mediated redox signaling in recipient cells	Melanoma	[129]
HSP72	Tumor-derived EVs	Immune suppression through IL-6/STAT3 activation in myeloid-derived suppressor cells	Multiple	[252]
PD-L1, CD20	DLBCL-derived EVs	Registered study of EV-associated PD-L1/CD20 and immunotherapy resistance	Diffuse large B-cell lymphoma	[NCT03985696]
VEGF, FGF, PDGF, bFGF, TGF-β, TNF-α, syndecan-4, GPC1, IL-8	Tumor-derived EVs	Angiogenesis	Multiple	[37]
VEGF90K	Breast cancer cell-derived EVs	Angiogenesis (VEGFRs activation)	Breast cancer	[131]
Dll4	Dll4-overexpressing U87 glioma cell-derived EVs	Vascular remodeling: Notch activation and capillary sprout retraction	Glioma cell-derived EVs; endothelial sprout model	[132]

GPC1, syndecan-4	Glioblastoma-derived EVs	Angiogenesis (endothelial proliferation/tube formation)	Glioblastoma	[133]
TIE2	Cervical cancer-derived EVs	Angiogenesis (macrophage TIE2-ANG2 pro-angiogenic signaling)	Cervical cancer	[138]
E-cadherin	Metastatic ovarian cancer-derived EVs	Angiogenesis	Metastatic ovarian cancer	[134]
DPP4	5-FU-resistant colon cancer cell-derived EVs	Angiogenesis (periostin upregulation via Twist1 and SMAD)	Colorectal cancer	[139]
Carbonic anhydrase 9	Renal cell carcinoma-derived EV	Angiogenesis	Renal cell carcinoma	[135]
Annexin A2 (ANXA2)	Breast cancer-derived EVs	Angiogenesis	Breast cancer	[136]
DEL-1	Mesothelioma cell-derived EVs	DEL-1 detected in EVs; angiogenesis-associated protein	Mesothelioma	[137]
Podoplanin	Tumor-derived EVs	Lymphangiogenesis	Multiple	[140]
Integrin αvβ5	Pancreatic cancer cell-derived EVs	Pre-metastatic niche formation (liver tropism via binding Kupffer cells)	Pancreatic cancer	[11]
Integrin α6β4, α6β1	Breast cancer cell-derived EVs	Lung pre-metastatic niche formation through uptake by lung fibroblasts and epithelial cells	Breast cancer	[11]
MET	Metastatic melanoma-derived EVs	Pre-metastatic niche formation (via MET pathway in bone marrow progenitor cells)	Metastatic melanoma	[145]
CD97	Gastric cancer-derived EVs	Pre-metastatic niche formation (via lymphatic pathway)	Gastric cancer	[146]
MIF	PDAC-derived EVs	Pre-metastatic niche formation (fibronectin induction via Kupffer cell TGF-β)	PDAC	[147]
EGFR	Gastric cancer-derived EVs	Liver pre-metastatic niche formation: miR-26a/b suppression and HGF upregulation in hepatic stromal cells	Gastric cancer	[148]
TGF-β, IL-6, CAV1	Tumor-derived EVs	EMT and ECM remodeling; pre-metastatic niche signaling	Multiple cancers	[142,150]
Annexin A2 (ANXA2)	Metastatic bladder cancer-derived EVs	EMT	Metastatic bladder cancer	[37]
MMP-13	Nasopharyngeal carcinoma-derived EVs	ECM remodeling and EMT (EMT promotion)	Nasopharyngeal carcinoma	[152]
LMP1	Nasopharyngeal carcinoma-derived EVs	EMT	Nasopharyngeal carcinoma	[154]
ITGB2	Papillary thyroid cancer-derived EVs	EV ITGB2 levels associated with EMT and lymph-node involvement	Papillary thyroid cancer	[155]
CD151, TSPAN8	Rat pancreatic adenocarcinoma-derived EVs	ECM degradation, stromal and hematopoietic-cell reprogramming, and EMT-associated gene expression	Rat pancreatic adenocarcinoma	[156]

Activities and associations are summarized in the experimental or clinical context of the cited studies. EV-associated proteins include membrane proteins and other proteins detected in the reported EV preparations. The Dll4 entry describes vascular remodeling in an endothelial sprout model. NCT identifiers denote registered clinical studies. 5-FU: 5-fluorouracil; ADAM10: a disintegrin and metalloproteinase 10; AKT1: protein kinase B alpha; ANG2: angiopoietin-2; ANXA2: annexin A2; B7-H3: B7 homolog 3; bFGF: basic fibroblast growth factor; CAV1: caveolin-1; CD8: cluster of differentiation 8; CD20: cluster of differentiation 20; CD44: cluster of differentiation 44; CD47: cluster of differentiation 47; CD97: cluster of differentiation 97; CD151: cluster of differentiation 151; DEL-1: developmental endothelial locus-1; DLBCL: diffuse large B-cell lymphoma; Dll4: delta-like ligand 4; DPP4: dipeptidyl peptidase 4; ECM: extracellular matrix; EGFR: epidermal growth factor receptor; EMT: epithelial-mesenchymal transition; EV: extracellular vesicle; FGF: fibroblast growth factor; GPC1: glypican-1; HCC: hepatocellular carcinoma; HGF: hepatocyte growth factor; HSP72: heat shock protein 72; IL-6: interleukin-6; IL-8: interleukin-8; ITGB2: integrin beta-2; ITGB4: integrin beta-4; LAMC1: laminin subunit gamma-1; LMP1: latent membrane protein 1; MAPK: mitogen-activated protein kinase; MET: hepatocyte growth factor receptor; MIF: macrophage migration inhibitory factor; miR-26a/b: microRNA-26a/b; MMP-13: matrix metalloproteinase 13; mTOR: mechanistic target of rapamycin; MUC1: mucin-1; NK: natural killer; NKG2D: natural killer group 2 member D; PD-L1: programmed death-ligand 1; PDAC: pancreatic ductal adenocarcinoma; PDGF: platelet-derived growth factor; PDGFR-β: platelet-derived growth factor receptor beta; PSGR: prostate-specific G protein-coupled receptor; RhoA: Ras homolog family member A; ROS: reactive oxygen species; SMAD: Sma- and Mad-related proteins; STAT3: signal transducer and activator of transcription 3; TGF-β: transforming growth factor beta; TIE2: tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 2; TNF: tumor necrosis factor; TNF-α: tumor necrosis factor alpha; TSPAN8: tetraspanin-8; Twist1: twist-related protein 1; VEGF: vascular endothelial growth factor; VEGF90K: 90-kDa vascular endothelial growth factor; VEGFA: vascular endothelial growth factor A; VEGFR: vascular endothelial growth factor receptor.

molecules also potentiate metastatic traits and therapeutic resistance. For example, the prostate-specific G protein-coupled receptor (PSGR) augments migration, stemness, and EMT[112]. ADAM10 on cancer-associated fibroblast exosomes activates Notch and Ras homolog family member A (RhoA) to boost motility[113]. Fibronectin 1 and laminin subunit gamma-1 (LAMC1) are associated with gastric cancer exosomes to facilitate peritoneal spread[114]. B7 homolog 3 (B7-H3) on colorectal cancer exosomes activates protein kinase B alpha (AKT1)/mechanistic target of rapamycin (mTOR)/vascular endothelial growth factor A (VEGFA) signaling to promote angiogenesis and metastasis[115]. Melanoma exosomal platelet-derived growth factor receptor beta (PDGFR-β) activates PI3K/AKT to bypass MAPK inhibition and foster resistance to targeted therapy[116]. Collectively, these findings implicate EV-associated proteins in organ targeting, metastatic reprogramming, and niche conditioning, alongside distinct cell-surface signaling mechanisms[117].

Exosomal surface proteins in immune evasion in cancer

Exosomes can suppress anti-tumor immunity through surface-mediated interactions with immune cells[118,119]. Exosomal cluster of differentiation 47 (CD47) engages signal-regulatory protein alpha (SIRPα) on phagocytes to deliver a "don't eat me" signal that blocks phagocytosis and aids immune escape[19]. Exosomal PD-L1 inhibits T-cell activation and can directly suppress cluster of differentiation 8 ($CD8^+$) T cells, hence weakening anti-tumor immunity[120]. On the contrary, reducing exosomal PD-L1 enhances anti-tumor responses[121]. Studies have shown that decorating exosomes with anti-programmed cell death protein 1 (PD-1) can activate T cells and inhibit tumor growth[122-125]. Furthermore, the immunosuppressive capacity of exosomal PD-L1 can be therapeutically beneficial in transplantation. Following Wharton's jelly mesenchymal stem cell (WJMSC) infusion, circulating small-vesicle PD-L1 increased and suppressed T-cell activity *in vitro*[126]. In a separate mouse heart-allograft model, engineered mesenchymal stem cell (MSC)-derived small EVs (sEVs) co-displaying fibrinogen-like protein 1 (FGL1) and PD-L1 prolonged graft survival[127]. Exosomes released from tumors carry transforming growth factor beta (TGF-β) on their surface, which downregulates natural killer group 2 member D (NKG2D) on natural killer (NK) and $CD8^+$ T cells, and, together with exosomal NKG2D ligands, suppresses rather than activates cytotoxicity and hence promotes immune escape[128]. Although exosomal TNF can activate NK cells in some conditions, melanoma exosomal TNF may also induce reactive oxygen species (ROS)-mediated T-cell dysfunction and facilitate cancer progression[129]. In diffuse large B-cell lymphoma (DLBCL), exosomal cluster of differentiation 20 (CD20) and PD-L1 can act as decoy receptors for therapeutic antibodies such as rituximab and immune checkpoint inhibitors. They contribute to immune escape and therapy resistance. This key finding is being evaluated in a clinical trial involving aggressive B-cell non-Hodgkin lymphoma [NCT03985696].

EV surface-mediated angio- and lymphangiogenesis

EV surface-associated signals modulate endothelial and stromal responses involved in angiogenesis and lymphangiogenesis. VEGF signaling is central to tumor angiogenesis[130]. In breast cancer-derived microvesicles, 90-kDa vascular endothelial growth factor (VEGF90K) is associated with surface HSP90 and stimulates endothelial VEGF receptor signaling and angiogenic responses[131]. Exosomal delta-like ligand 4 (Dll4) activated Notch signaling in adjacent endothelial cells and induced capillary-sprout retraction[132]. Glioblastoma exosomes enriched in glypican-1 (GPC1) and syndecan-4 stimulate endothelial proliferation and tube formation, supporting aggressive tumor growth[133]. Exosomal E-cadherin from metastatic ovarian cancer further promotes new vessel formation[134]. Additional surface proteins such as carbonic anhydrase 9 and annexin II augment angiogenic programs[135,136]. Developmental endothelial locus-1 (DEL-1) has also been detected in mesothelioma-derived EV preparations, although its external exposure and EV-associated angiogenic activity were not established in that study[137]. Macrophages also participate in angiogenesis. Cervical cancer exosomes deliver tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 2 (TIE2) to macrophages to increase their interaction with angiopoietin-2 (ANG2) and hence amplify pro-angiogenic signaling[138]. Besides, in 5-fluorouracil (5-FU)-resistant colon cancer, exosomal

dipeptidyl peptidase 4 (DPP4) upregulates periostin in endothelial cells by inducing twist-related protein 1 (Twist1) nuclear translocation and activating Sma- and Mad-related proteins (SMAD) signaling, thereby driving angiogenesis[139]. Beyond blood vessels, exosomal podoplanin also promotes lymphangiogenesis, expanding lymphatic routes for metastatic dissemination[140].

Pre-metastatic niche establishment by organotropic EVs

Pre-metastatic niche formation is very important in cancer cell metastasis; it can be facilitated by EV-associated proteins through tissue-specific uptake and stromal reprogramming[141]. For example, TGF-β and CAV1 on tumor exosomes initiate niche formation by modulating cellular interactions and inflammatory tone[142,143]. Integrin repertoires can support preferential interactions with recipient cells. In the reported tumor models, exosomal αvβ5 was implicated in uptake by liver Kupffer cells, whereas α6β4 and α6β1 were associated with interactions with lung fibroblasts and epithelial cells and with organ-associated niche formation[11,144]. Metastatic melanoma exosomes that carry MET can activate MET signaling in bone marrow progenitor cells, increasing vascular leakiness and inflammation while enhancing pro-metastatic behavior[145]. Besides, exosomal CD97 facilitates niche preparation and lymphatic dissemination[146]. In pancreatic ductal adenocarcinoma (PDAC), EV-associated macrophage migration inhibitory factor (MIF) activates Kupffer cells to induce TGF-β, which drives fibronectin deposition and creates an immunosuppressive, tumor-permissive microenvironment[147]. The gastric cancer exosomal EGFR suppresses miR-26a/b in hepatic stromal cells, derepressing hepatocyte growth factor and establishing a liver microenvironment conducive to metastatic seeding[148]. Taken together, EV-associated proteins can contribute to metastatic niche formation in multiple cancer models.

EV surface proteins in extracellular matrix remodeling and EMT

EMT is a fundamental biological process that cancer cells hijack to drive invasion and dissemination, thereby facilitating metastasis. EV-associated proteins, including surface-exposed mediators, can remodel the extracellular matrix (ECM) and induce EMT, thereby enhancing motility and invasiveness in target tissues[149]. Interestingly, studies found that exosomal TGF-β and interleukin-6 (IL-6) are potent EMT inducers[150]. Besides, annexin A2 participates in EMT in metastatic bladder cancer[37], and metastatic breast cancer exosomal HSP90 activates matrix metalloproteinase 2 (MMP-2) to remodel ECM and promote invasion[151]. In nasopharyngeal carcinoma, exosomal matrix metalloproteinase 13 (MMP-13) drives EMT[152], consistent with the broader role of MMP activation in releasing matrix-bound growth factors during ECM degradation to facilitate metastasis[153]. Furthermore, the nasopharyngeal carcinoma exosomal LMP1 further triggers EMT and enhances migration and invasion[154]. The elevated exosomal integrin beta-2 (ITGB2) also correlates with EMT extent and lymph node involvement[155]. Other exosomal surface proteins such as the tetraspanins cluster of differentiation 151 (CD151) and TSPAN8 form complexes with integrins and proteases to drive ECM degradation, while reprogramming hematopoietic cells and inducing pro-inflammatory cytokines that help sustain a pro-metastatic niche[156].

PHYSIOLOGICAL AND PATHOLOGICAL SIGNIFICANCE OF EV SURFACE-MEDIATED COMMUNICATION IN IMMUNE-RELATED DISEASES

EV-mediated communication may influence immune niches where vesicles modulate inflammation, antigen presentation, and lymphocyte survival. In physiology, exosome-mediated communication supports interactions between epithelial barriers, dendritic cells (DCs), and lymphoid tissues to coordinate host defense[157]. In pathology, these surface-mediated interactions can sustain chronic inflammation, drive T-cell exhaustion in persistent infections, or enforce immunosuppression that can be either therapeutically desirable in transplantation or detrimental in settings of impaired anti-pathogen responses. Through these mechanisms, exosomal surface proteins influence inflammatory disorders, transplant rejection, viral infections, and autoimmune disease trajectories, as summarized in Table 3.

Table 3. EV-associated proteins involved in immune modulation

EV-associated protein(s)	Disease or biological process	Reported activity or study focus	References
Fibronectin	Inflammation	Trophoblast-derived EV fibronectin induces macrophage IL-1β production	[159]
LFA-1	Immune activation	LFA-1 is carried by mast-cell EVs that stimulate B- and T-cell proliferation and cytokine production	[160]
Bat-3	HIV-1 infection; chronic inflammation	Registered study of Bat-3/HIV-1 antisense protein-associated EVs and NK-cell activity	[NCT05243381]
MHC-I, MHC-II	T-cell activation	MHC-II-associated $CD4^+$ T-cell activation and MHC-I-associated $CD8^+$ T-cell priming	[19,161-163]
CD9, CD81, CD82, A33 antigen	Antigen presentation, immune activation	Lower antigen presentation threshold	[54,55]
CD80, CD86	T-cell activation	Activate T cells	[19]
ICAM-1	Immune activation	Stimulates T cells indirectly	[83]
PD-L1	Immune suppression; transplantation-related models	PD-L1-bearing MSC EVs suppress T-cell activity; engineered FGL1/PD-L1 EVs prolong graft survival in mice	[126,127]
CD13	Local neuropeptide metabolism	Microglial EV-associated CD13 cleaves neuropeptides	[165]
CD24	Hyperinflammation in ARDS and COVID-19	Registered studies evaluating CD24-overexpressing EVs for safety and prevention of clinical deterioration	[NCT05947747, NCT04902183, NCT04747574, NCT04969172]
CD63	Ocular inflammation and immune privilege	CD63-associated EV release from ocular epithelia during inflammation; proposed contribution to ocular immune privilege	[166]

Bat-3 is an alternative name for BAG6 (B-cell lymphoma 2-associated athanogene 6). NCT identifiers refer to registered studies in ClinicalTrials.gov; A33 is an antigen designation. ARDS: Acute respiratory distress syndrome; Bat-3: human leukocyte antigen B-associated transcript 3; CD4: cluster of differentiation 4; CD8: cluster of differentiation 8; CD9: cluster of differentiation 9; CD13: cluster of differentiation 13; CD24: cluster of differentiation 24; CD63: cluster of differentiation 63; CD80: cluster of differentiation 80; CD81: cluster of differentiation 81; CD82: cluster of differentiation 82; CD86: cluster of differentiation 86; COVID-19: coronavirus disease 2019; EV: extracellular vesicle; FGL1: fibrinogen-like protein 1; HIV-1: human immunodeficiency virus type 1; ICAM-1: intercellular adhesion molecule 1; IL-1β: interleukin-1 beta; LFA-1: lymphocyte function-associated antigen 1; MHC-I: major histocompatibility complex class I; MHC-II: major histocompatibility complex class II; MSC: mesenchymal stem cell; NK: natural killer; PD-L1: programmed death-ligand 1.

Surface-mediated EV immune activation in tissue contexts

Exosomal surface proteins can amplify inflammation in a tissue-specific manner by docking to extracellular matrices and leukocytes within inflamed microenvironments[158]. The exosomal fibronectin directly induces interleukin-1 beta (IL-1β) production that enhances inflammation in the target tissues[159]. LFA-1 on exosomes reinforces leukocyte activation through inflammatory signaling pathways, which recruit immune cells and promote effector function at sites where exosomes accumulate[160]. In chronic viral infection, exosome-mediated signaling may perpetuate pathological inflammation. A clinical trial has been conducted to examine whether the exosomal surface protein human leukocyte antigen B-associated transcript 3 (Bat-3) binds the human immunodeficiency virus type 1 (HIV-1) antisense protein (ASP) and interferes with natural killer cell protein 30 (NKp30)-dependent NK cytotoxicity [NCT05243381]. This study may clarify the relationships among viral control, sustained chronic immune activation, T-cell exhaustion, and poor immune reconstitution. Targeted delivery of antigen-presenting machinery further couples epithelial and myeloid compartments to lymphoid niches. Dendritic cell-derived exosomes (Dex) bearing MHC-II activate cluster of differentiation 4 $(CD4)^+$ T cells, while those displaying MHC-I support $CD8^+$ T-cell priming, extending antigen presentation beyond the confines of the cell membrane[19,161,162]. It has been reported that B-cell-derived exosomes also present MHC-I and MHC-II that can augment T-cell responses, thereby significantly broadening the network of cells capable of initiating adaptive immunity[163]. The regulatory effects on immune cells are further enhanced by costimulatory and adhesion molecules on exosomes. For

example, DC exosomal cluster of differentiation 80 (CD80) and cluster of differentiation 86 (CD86) promote T-cell activation[19], and ICAM-1 facilitates efficient immune synapse formation and signaling[83]. At mucosal interfaces, local vesicle-mediated communication is evident. Intestinal epithelial cell-derived exosomes enriched in CD9, CD81, cluster of differentiation 82 (CD82), and the A33 antigen communicate with DCs, which can lower antigen presentation thresholds and trigger rapid immune responses[54]. The basolateral exosomes bearing A33 antigen traverse the porous basal membrane to coordinate immune communication across the mucosa[55]. Taken together, these observations illustrate how exosomal surface proteins support antigen presentation and T-cell activation within specific anatomical niches.

Surface-mediated EV immune suppression in tissue contexts

Exosomal surface proteins can mediate immunosuppression to preserve tissue integrity or promote tolerance[164]. PD-L1-bearing small EVs from WJMSCs suppressed T-cell activity *in vitro*[126]. Microglia-derived exosomes displayed active cluster of differentiation 13 (CD13) capable of degrading neuropeptides, indicating a potential role in local neuropeptide metabolism[165]. Cluster of differentiation 24 (CD24)-overexpressing exosomes have been investigated for anti-inflammatory effects in acute respiratory distress syndrome (ARDS) and severe coronavirus disease 2019 (COVID-19) [NCT05947747, NCT04902183, NCT04747574, NCT04969172], aiming to evaluate their safety and efficacy in preventing clinical deterioration. These examples illustrate how EV-associated surface signals modulate immune responses and local signaling.

EV surface-mediated maintenance of immune privilege

Exosome-mediated communication also preserves immune privilege in barrier tissues. Studies using ARPE-19 cells identified CD63-associated EV release and proposed a possible role for these vesicles in ocular immune privilege[166]. Similar regulatory architectures are likely to operate in other immune-privileged sites such as the brain and placenta, where exosomal surface proteins allow finely tuned crosstalk across anatomical barriers to balance necessary surveillance with the prevention of immunopathology.

PHYSIOLOGICAL AND PATHOLOGICAL SIGNIFICANCE OF EV SURFACE-MEDIATED COMMUNICATION IN OTHER DISEASES

Exosomal surface proteins participate in neural, cardiovascular, and skeletal communication beyond cancer and immunity, with potential roles in disease onset, progression, and repair[167]. By shaping docking, uptake, and signaling within specific tissues, these surface proteins may influence whether exosomes propagate pathology or provide protection in these diseases.

Neural EV transfer and prion propagation

In neurodegeneration, EV-mediated transfer can contribute to spread within neuronal and glial networks. In transmissible spongiform encephalopathies, exosomes that carry normal cellular PrP can foster its conversion to the pathogenic scrapie isoform of PrP (PrPSc), and then transfer this misfolded species to uninfected neuronal and non-neuronal cells, initiating and propagating prion pathology within neural networks[168]. This transfer shows that EV-associated components can increase uptake in neural cells, turning normal protein trafficking into a route for neurodegenerative propagation.

Cardiac and vascular EV signaling in ischemia and cardiovascular diseases

Exosomes interacting with cardiomyocytes and vascular endothelium can activate protective or reparative programs through surface protein-mediated engagement of cardiomyocytes and immune cells. During ischemia-reperfusion, exosomal heat shock protein 70 (HSP70) engages Toll-like receptor 4 (TLR4) on cardiomyocytes to initiate a cytoprotective cascade culminating in HSP27 activation, thereby increasing cell survival by reducing damage under stress[169]. Following cerebral ischemia, exosomes from brain

microvascular endothelial cells showed altered surface-protein patterns, including cluster of differentiation 146 (CD146), ephrin type-A receptor 2 (EPHA2), adenosine triphosphate-binding cassette subfamily B member 5 (ABCB5), and ITGB2[170].

Skeletal EV signaling and bone repair

Within the bone microenvironment, EV-associated signals can influence osteoclast and osteoblast responses involved in remodeling and regeneration. CD9 deficiency reduced exosome production and delayed fracture healing in mice; MSC-derived exosomes partially rescued repair[171]. These findings support a role for MSC-derived exosomes in fracture repair, while the contribution of specific surface proteins to skeletal targeting and their therapeutic relevance to osteoporosis require further evaluation.

EV-ASSOCIATED SURFACE PROTEINS AS CANDIDATE DISEASE BIOMARKERS

Surface proteins that contribute to exosome recognition and tissue distribution also form disease-associated profiles that can be sampled noninvasively from biofluids[172]. These profiles may contain information about donor-cell states and disease processes, supporting their investigation for diagnosis, prognosis, and response monitoring across cancer and nonmalignant disorders. Elevated or altered expression levels of defined exosomal surface proteins can serve as promising candidate circulating biomarkers[173].

Diagnostic readouts of disease-associated pathology

Multiple EV-associated proteins exhibit disease-associated changes that are being investigated for diagnostic use[174]. For example, in non-small cell lung cancer (NSCLC), a multiplex EV protein panel distinguished patients with advanced disease from patients with suspected but unconfirmed lung cancer in a cross-validated study[175]. Proteomic analysis of pleural-effusion EVs provided additional candidate proteins[176]. TSPAN8 expression was associated with gastric cancer progression in tissue and experimental EV studies[110]. In breast cancer, cell-line EV proteomics identified candidate proteins including GLUT-1, GPC1, and ADAM10[177], while CD24 was detected in patient-derived EV preparations[178]. Tetraspanin profiles are also under investigation. Increased EV-associated CD63 was reported in melanoma patient samples[179], while prostate and breast cancer cell-line studies showed variable CD63 expression across EV preparations[180]. CD63 is a broadly expressed EV marker, so these findings do not establish a cancer-specific diagnostic signal. In lung cancer, patients display increased exosomal cluster of differentiation 171 (CD171), CD151, and tetraspanin expression[181]. Among GPI-anchored proteins, exosomal GPC1 was reported to detect early-stage PDAC and discriminate malignant pancreatic cancer from chronic pancreatitis[182,183]. In melanoma, caveolae-associated CAV1 is elevated in exosomes from patients[179]. EV-associated receptors and cytokines provide further candidate signals. EGFR and TGF-β were detected in serum EV preparations from brain tumor patients[184], and elevated EV-associated EGFR was reported in five of nine lung cancer patients in a pilot study[185]. Other proteins such as human epidermal growth factor receptor 2 (HER2) and C-C motif chemokine receptor 6 (CCR6) are increased on exosomes from gastric cancer patients[98,186]. Cell adhesion and tight junction proteins are similarly informative. EV-associated CD24 and claudin-4 have been investigated as candidate ovarian cancer markers in patient cohorts and pilot assays[187,188], while exosomal CD44 and tumor-associated calcium signal transducer 2 (TACSTD2) increase in bladder cancer, alongside cluster of differentiation 36 (CD36), basigin, and cluster of differentiation 73 (CD73) on tumor-derived vesicles[98,189]. Tumor-associated carbohydrate antigens captured on vesicles, including carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3), and trophoblast glycoprotein (5T4), increase with disease progression in breast and bladder cancers[189,190]. Interestingly, immune checkpoint profiling reveals that exosomal PD-L1 is elevated in head and neck squamous cell carcinoma, PDAC, and gastric cancer[191-193]. Heat shock proteins (HSPs) are also informative. HSP27, heat shock protein 60 (HSP60), and HSP90 were detected by Western blotting in plasma EV preparations from patients with thyroid cancer[194]. A separate study protocol proposed evaluating circulating EV-associated HSP70 in patients with solid tumors, including

ovarian cancer[195]. Higher serum EV-associated gamma-glutamyltransferase activity was reported in prostate cancer than in benign prostatic hyperplasia (BPH), supporting investigation of its diagnostic potential[196]. Urinary EV-associated leucine-rich alpha-2-glycoprotein 1 (LRG1) was identified as a candidate NSCLC marker[197], and EV-associated fetuin-A was investigated as a candidate acute kidney injury marker in animal models and a small patient series[198].

Several EV-associated proteins show disease-associated changes in abundance or detectability[199]. CD81 in the serum exosome-enriched fraction was elevated in patients with chronic hepatitis C and elevated alanine aminotransferase (ALT)[200]. Plasma EV α-synuclein differed between patients with Parkinson's disease and comparison groups in two cohorts[201]; epidermal growth factor receptor variant III (EGFRvIII) mRNA was detected by nested RT-PCR in serum EV preparations from patients with glioblastoma[202], and exosomal PrPSc was detected in transmissible spongiform encephalopathies[168]. These disease-associated signatures provide a basis for developing EV-associated proteins and RNA as candidate circulating biomarkers[203].

Studies of EV-associated protein markers have reported diagnostic performance in defined patient cohorts. The original exosomal GPC1 study reported 100% specificity for PDAC in its study cohort[183]. A separate serum study reported specificities of 79.49% for carbohydrate antigen 19-9 (CA19-9) and 82.14% for serum GPC1[204]. These estimates describe performance within the respective study populations and analytical settings. An EV-associated DEL-1 study reported 94.7% sensitivity and 86.36% specificity for breast cancer detection in its test cohort, with corresponding estimates of 92.31% and 86.62% in a separate validation cohort[205]. Serum EV-fraction CEA has also been investigated for its clinical associations in colorectal cancer[206].

Reported findings for GPC1 include associations with tumor burden and postoperative changes, together with variation in diagnostic discrimination across study cohorts[207,208]. Cohort composition, comparator populations, sample handling, EV isolation, antibodies, and detection platforms provide the methodological context for interpreting these findings. Clinical development of EV-associated protein biomarkers involves standardized analytical workflows and evaluation in independent cohorts with clinically relevant comparator groups. Biofluids contain EVs from multiple cell types and vesicle subpopulations, while shared surface proteins can limit assignment of tissue origin and disease specificity[3,209,210]. Candidate discovery, diagnostic validation for a defined clinical use, and routine clinical implementation therefore represent distinct stages; the studies reviewed here include exploratory findings and cohort validation but do not establish routine clinical use.

Dynamic markers of disease progression and invasion

EV-associated proteins are also being investigated as indicators of disease progression[211]. In pancreatic cancer, exosomal GPC1 correlates with disease burden. Its levels have been associated with tumor size and metastatic load[183,207]. Elevated exosomal PD-L1 is found to be associated with aggressiveness of head and neck squamous cell carcinoma and the extent of lymph node invasion[191]. Cellular and tumor-tissue ITGB4 expression has been associated with triple-negative breast cancer aggressiveness, providing biological context rather than direct evidence for an EV biomarker[212]. In patients with chronic hepatitis C, higher CD81 levels in the serum exosome-enriched fraction were associated with severe liver fibrosis[200]. CD24 was detected in EVs from ovarian cancer ascites, but its detection was not correlated with tumor-tissue CD24 expression in that study[213]. However, not all markers increase with malignancy. Exosomal survivin-2B, which counteracts pro-tumor survivin and survivin-ΔEx3 and promotes cancer cell death, exhibits a negative correlation with tumor grade, supporting further investigation as a candidate marker of disease severity[214].

Prognosis and treatment monitoring through EV-associated protein signatures

Exosomal surface proteins have been investigated as possible correlates of outcomes and treatment response. In gastric cancer, co-expression of MHC-I with exosomal PD-L1 promotes immune exhaustion through T-cell suppression, linking higher exosomal PD-L1 levels with poorer immune status and worse survival[193]. In pancreatic cancer, EV-associated GPC1 levels and their postoperative changes were associated with survival in a longitudinal patient cohort[183]. Detection of CD24 in ovarian cancer ascites EVs did not establish an EV-based survival association[213]. Survivin-2B remains a candidate prognostic marker requiring longitudinal validation[214].

EV-associated protein profiles can change in response to treatment[215]. Studies show that patients with colorectal cancer exhibit marked reductions in exosomal GPC1 after surgery, with decreases exceeding the baseline differences between patients and healthy controls[216]. Postsurgical thyroid cancer patients exhibit notable declines in exosomal HSP27, HSP60, and HSP90 compared with preoperative levels[194]. These changes suggest that EV-associated protein measurements may inform longitudinal treatment monitoring; their relationship to therapeutic efficacy requires validation.

THERAPEUTIC APPLICATIONS OF SURFACE-ENGINEERED EVs

Native exosomal surface proteins mediate docking and uptake across tissues. However, compared with synthetic carriers, the intrinsic targeting ability of exosomes is less selective. Moreover, exosomes can be cleared rapidly *in vivo*, limiting their therapeutic efficacy. Engineering the exosomal surface to display defined ligands, receptors, and peptides can improve target-cell engagement and delivery in specific preclinical models[217,218]. Tissue accumulation, cellular uptake, functional cargo delivery, and therapeutic efficacy are distinct outcomes: accumulation or uptake alone does not establish cargo activity or treatment benefit[3].

Surface-engineered EV delivery of chemotherapeutics

Surface engineering has been explored to improve EV targeting and drug delivery in tumor and ischemia models[219]. In one study, fusion of the exosomal scaffold lysosome-associated membrane protein 2 isoform B (LAMP2B) with the αv integrin-binding internalizing arginine-glycine-aspartic acid peptide (iRGD) on Dex-directed exosomes to αv integrin-positive breast cancer cells for therapeutic delivery of doxorubicin[220]. In a transient middle-cerebral-artery-occlusion model, exosomes chemically conjugated with the cyclic arginine-glycine-aspartic acid-D-tyrosine-lysine peptide c(RGDyK) targeted ischemic brain lesions, and curcumin-loaded vesicles reduced inflammation and apoptosis[221]. These results support enhanced targeting of ischemic brain lesions and local anti-inflammatory and anti-apoptotic effects.

Engineering EV recognition for siRNA therapeutics

The engineered exosomes can also deliver siRNA for therapy[222,223]. Exosomes expressing a fusion of LAMP2B with designed ankyrin repeat protein (DARPin) G3 can bind to HER2/Neu on breast cancer cells and deliver tumor protein D52 (TPD52)-targeting siRNA that achieved 70% knockdown in SKBR3 cells[224]. LAMP2B fused to iRGD can bring siRNA-loaded exosomes to lung tumors to knock down Kirsten rat sarcoma viral oncogene homolog (KRAS) and suppress tumor growth[225]. Fusing LAMP2B with interleukin-3 (IL-3) targets the IL-3 receptor on chronic myeloid leukemia cells, enabling exosomes to deliver breakpoint cluster region-Abelson tyrosine kinase (BCR-ABL) siRNA to suppress chronic myeloid leukemia progression[226]. For central nervous system delivery, dendritic cell-derived exosomes displaying LAMP2B fused to a rabies virus glycoprotein (RVG) peptide delivered GAPDH siRNA to neurons, microglia, and oligodendrocytes. In a separate experiment, siRNA targeting beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) reduced its mRNA and protein in the brains of wild-type mice, demonstrating functional RNAi delivery to a target relevant to Alzheimer's disease[227]. These studies show

that engineered surface ligands can support recipient-cell engagement and functional siRNA delivery, with therapeutic benefit demonstrated only in models where disease outcomes were directly evaluated.

miRNA delivery through ligand-directed EV targeting

Engineered exosomes can reprogram tumor and hepatic niches by delivering therapeutic microRNAs to recipient cells[228]. For example, a platelet-derived growth factor receptor (PDGFR)-GE11 fusion enhances binding to EGFR-positive breast cancer, allowing exosomal transfer of let-7a miRNA and consequent tumor growth inhibition[229]. Exosomes displaying a CD63-apolipoprotein A-I fusion bound scavenger receptor class B type 1 on HepG2 cells and delivered miR-26a, reducing proliferation and migration *in vitro*[230]. These designs exploit receptor-ligand biology to improve recipient-cell engagement and cargo delivery in specific preclinical models.

Engineered EVs for regenerative tissue targeting

Surface engineering can steer exosomes to injured musculoskeletal and cardiac tissues to enhance repair[231]. In the knee joint, LAMP2B fused with MSC-binding peptide E7 on exosomes can deliver small-molecule cargo such as kartogenin (KGN) to promote chondrogenic differentiation and cartilage regeneration[232]. Besides, ischemic myocardium-targeting peptide (IMTP)-expressing exosomes can promote vasculogenesis and reduce fibrosis and inflammation[233]. Furthermore, cardiosphere-derived exosomes displaying a cardiomyocyte-targeting peptide (WLSEAGPVVTVRALRGTGSW) fused to LAMP2B showed increased myocardial retention 2 h after intramyocardial injection in non-infarcted mice and anti-apoptotic effects in cultured cardiomyocytes[234]. These studies show that engineered surface motifs can improve tissue-associated retention or recipient-cell engagement in selected preclinical models[235,236].

Surface protein engineering for immunomodulation and vaccination

Rewriting immune interactions at disease sites can also be achieved by modifying exosomes with immunologically active surface proteins. Given that the well-known CD47-SIRPα engagement delivers a "don't eat me" signal to macrophages that protects tumor cells[237], SIRPα overexpressed on exosomes can act as a decoy to disrupt this axis and reduce tumor resistance to phagocytosis, thereby enhancing innate clearance through a surface-mediated interaction rather than requiring intracellular cargo delivery[238]. For vaccination, a DNA construct encoding a CD63-ovalbumin (OVA) fusion generated OVA-bearing EVs in transfected cells; purified EVs induced antigen-specific T-cell responses. Separately, vaccination with the pCD63-OVA DNA construct elicited OVA-specific $CD8^+$ T-cell responses and inhibited tumor growth in mice[239]. Fusing exosomal scaffolds such as LAMP2B, PDGFR, and CD63 to peptides, cytokine domains, and high-affinity binders can improve recipient-cell engagement and delivery in preclinical models. Surface stability, reproducible biodistribution, and clearance remain challenges for translation[16,240].

Recent studies extend these foundational engineering approaches. Cardiomyocyte-binding-peptide-engineered EVs improved cardiac targeting, retention, and functional outcomes after intravenous delivery in a mouse myocardial-infarction model[241]. A self-cleaving mini-intein loading system combined with fusogenic vesicular stomatitis virus glycoprotein G (VSV-G) improved functional intracellular delivery of proteins and genome-editing cargoes; brain recombination followed intracerebroventricular administration[242].

Administration route and dose shape circulation, tissue exposure, and clearance[12,16]. Free label or transfer of labeled membrane components can influence measured biodistribution[3,12]. During scale-up, purification, and storage, maintaining surface ligand display, biological potency, and batch consistency remains a manufacturing challenge[16,243]. Minimal Information for Studies of Extracellular Vesicles 2023 (MISEV2023) provides guidance for vesicle characterization and functional controls, while clinical development also depends on validated manufacturing processes and indication-specific evaluation[3,16,244].

FUTURE PROSPECTS AND CHALLENGES

Resolving EV heterogeneity for surface-guided recognition and delivery

The surface "GPS" codes contribute to recipient-cell recognition and targeting as part of a multifactorial system, with potential applications in disease detection and drug delivery. Biodistribution also reflects vesicle origin, physicochemical properties, administration route, vascular accessibility, and clearance by mononuclear phagocytes; labeling, isolation, dose, and observation time influence its measurement[12]. An atlas linking EV biogenesis pathways, experimentally characterized surface features, and cell or tissue interactions could help identify context-dependent recognition patterns and guide their evaluation for diagnostic and therapeutic use.

Translating a surface-recognition framework into diagnostic and therapeutic applications requires evidence for each proposed clinical use. On the diagnostic front, we still have to resolve the issue of exosomal heterogeneity[245]. Single-vesicle and spatially resolved proteomics, paired with machine learning, may help investigate relationships between EV surface profiles, tissue distribution, and donor-cell states, provided that inferred signatures are validated in relevant samples. Longitudinal sampling and dynamic tracking are other strategies to link the exosomal surface complexity with treatment responses, disease progression, and prognosis. Although modular surface engineering offers a path to programmable recognition and delivery, additional strategies such as aptamers could refine cell- and tissue-specific recognition for drug delivery. Besides, microenvironment-responsive motifs could refine delivery to hypoxic, inflamed, or fibrotic niches. Addressing pharmacology is also essential. Strategies to extend circulation, reduce opsonization and rapid clearance, and enhance endosomal escape could improve functional delivery.

Disrupting pathological EV tissue targeting and recipient-cell interactions

Another therapeutic strategy is to disrupt tissue targeting or recipient-cell interactions of pathogenic exosomes. Strategies such as blocking exosomal adhesion modules, neutralizing pathogenic vesicle populations, inhibiting specific biogenesis routes, or transiently depleting circulating exosomes may prevent niche conditioning, angiogenesis, and immune escape in cancer and chronic inflammation.

CONCLUSION

Exosome biogenesis shapes cargo sorting and the surface protein repertoire, which contributes to recipient-cell recognition and receptor engagement and, together with other vesicle and host factors, influences tissue distribution in physiology and disease. Surface profiles are being investigated as noninvasive biomarkers for detection, prognosis, and treatment monitoring, with clinical value dependent on assay validation and disease specificity. Surface engineering has improved targeting or biological effects in selected preclinical models, but tissue accumulation, cellular uptake, functional cargo delivery, and therapeutic efficacy require distinct evidence. By connecting biogenesis and recognition mechanisms with these diagnostic and therapeutic evidence requirements, this review provides a framework for interpreting EV surface proteins and prioritizing further validation.

DECLARATIONS

Authors' contributions

Performed the literature search: Fong HL, Leung CY, Qin T, Jiang W, Yang H, Ye Y, Peng M, Wang J
Prepared the original draft and figures and tables: Fong HL, Leung CY, Qin T
Conceived and designed the review: Xu K, Kwan HY
Critically revised and edited the manuscript: Qin T, Jia B, Xu K, Kwan HY
All authors read and approved the final version of the manuscript.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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