

Combination of extracellular vesicles and organoids as a prospective model for cancer research (Review)

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Received June 12, 2025; Accepted December 12, 2025

DOI: 10.3892/ol.2026.15476

Abstract. Malignant tumors remain a major global health challenge. Despite substantial improvements in survival rates for patients with cancer, a translational bottleneck persists, which hinders the clinical application of abundant experimental and preclinical findings. This issue reflects the inherent complexity and heterogeneity of tumors and highlights the urgent need for more clinically predictive tumor models. Over the past decade, growing evidence has highlighted the pivotal roles of extracellular vesicles (EVs) and organoids in cancer research. EVs function as stable carriers of intercellular communication, transporting key signaling molecules that regulate tumor growth, migration and angiogenesis. Organoids are three-dimensional (3D) cell culture models grown in an extracellular matrix that can be co-cultured with different cell types to mimic complex cellular interactions within a 3D environment. Increasingly, organoid and other 3D culture models are being used to study the physiological and pathological functions of EVs. In the present review, the classification, characteristics and functions of EVs in oncology are systematically outlined and the application of organoid models in cancer therapeutics are highlighted. Furthermore, the integration of organoids with EVs-based approaches is explored as an emerging research direction in oncology. Finally, the challenges and future opportunities for combined organoid-EVs models are discussed. The review aims to provide insights into

organoids and EVs that may help to drive innovation in the development of cancer treatment strategies.

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1. Introduction

Cancer remains a major global health concern and a leading cause of mortality worldwide. It is characterized by uncontrolled cell proliferation, driven by cancer cells that have developed novel mechanisms to regulate their own growth and differentiation (1). Tumor development and metastasis are sustained by complex interactions within the tumor microenvironment (TME), involving both cancer-cell-intrinsic factors and stromal components. Therefore, elucidation of these intercellular communication networks is essential for understanding tumor biology (2).

Extracellular vesicles (EVs) are now recognized as crucial mediators of cell-to-cell communication in cancer. Substantial evidence demonstrates that EVs are secreted by virtually all cells within the TME and facilitate the transfer of various biological macromolecules. As specialized carriers of intercellular signals, EVs contribute to critical oncogenic processes such as cell proliferation, metastasis and epithelial-mesenchymal transition. Given their multifaceted roles in the modulation of tumor behavior, EVs show great promise as clinical biomarkers and novel platforms for cancer therapeutics, paving the way for improved diagnostic and treatment strategies (3,4).

Previous research on EVs has largely utilized two-dimensional (2D) monolayer cultures, with EVs derived from three-dimensional (3D) models remaining relatively underexplored. Organoids, as *in vitro* 3D culture systems, recapitulate the complex architecture and biological processes

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Key words: extracellular vesicles, organoid, 3D culture, cancer research

of *in vivo* tumors. This makes them a powerful tool for cancer research and provides a novel option for clinical studies in tumor biology (5,6). The complexity of the TME is fueling growing interest in organoid-derived EVs. These models hold considerable potential for simulating tumor-specific intercellular communication and warrant further investigation (7,8). Research into the clinical applications and challenges of integrating organoids with EVs is expected to contribute to the development of tumor biobanks, novel therapeutic strategies and precision oncology.

In the present review, the biogenesis and isolation methods of EVs are delineated, and their advantages and functions in tumor research are described. In addition, the potential of organoids as promising models for investigating the TME is discussed. Furthermore, recent advances in EVs derived from tissue organoids are summarized and the prospects and challenges of integrating organoid and EVs models for cancer therapy are discussed.

2. EVs

Origin and classification of EVs. EVs have been extensively studied over the past few decades, but their therapeutic applications have emerged more recently. Understanding the history of EV discovery is essential for appreciating their biological importance and for guiding the development of new therapeutic strategies. The discovery of EVs dates back to 1946, when Chargaff and West (9) identified platelet-derived particles, termed 'platelet dust', in the blood. This was followed by the identification of cell-derived vesicles in mouse cartilage by Anderson in 1969 (10). In 1996, Raposo *et al* (11) demonstrated that B-cell-derived exosomes contain MHC class II molecules and can directly induce T-cell responses. Subsequent studies revealed that T cells release EVs carrying bioactive ligands, such as Fas and TNF-related apoptosis-inducing ligand, indicating a crucial role in immune regulation. EVs perform diverse immune functions by interacting with recipient cells via their surface proteins or by delivering bioactive cargo, thereby altering the functional properties of the recipient cells (12). Unlike classical signaling mechanisms based on direct cell contact or secreted factors, EVs provide a distinct mode of intercellular communication, with the ability to transfer proteins and RNA to directly modulate cellular functions (13). These findings have highlighted the crucial importance of EVs in cell signaling and suggested new strategies for disease diagnosis and treatment.

EVs are phospholipid bilayer-enclosed particles, which are released by diverse cell types under both physiological and pathological conditions and have been detected in tissues and bodily fluids, including serum, cerebrospinal fluid, saliva and urine (14). Cells package signaling molecules, including proteins, lipids and nucleic acids, into EVs. This packaging protects the molecules from degradation and enables them to evade immune surveillance, thereby facilitating local and long-distance intercellular communication (15).

Recent advances in EV isolation and characterization have led to the discovery of a growing number of EV subtypes. Most mammalian cells, which typically measure 10-100 μm in diameter, release a heterogeneous population of EVs, along with non-vesicular extracellular nanoparticles (NPs). The

overlapping size ranges of EVs and NPs makes it challenging to differentiate between them. Well-characterized EVs types include exosomes, which are generated via the endocytic pathway and are released into the extracellular space when multivesicular endosomes fuse with the plasma membrane; microvesicles, which form through the direct outward budding and fission of the plasma membrane; and apoptotic bodies, which are released from cells during programmed cell death, specifically apoptosis (16). Among these, the term exosome is the most widely used and studied. However, due to a lack of well-defined, category-specific markers, exosomes and microvesicles can typically only be definitively distinguished by confirmation of their distinct biogenesis pathways, often using techniques such as cryo-transmission electron microscopy (17).

EVs are classified into six main types based on their biogenesis, size and molecular markers. Exosomes, 30-150 nm in diameter, are formed within multivesicular vesicles (MVBs) through the endosomal pathway and are released upon MVB fusion with the plasma membrane. The main molecular markers for exosomes are tetraspan proteins (such as CD9, CD63 and CD81), tumor susceptibility gene 101 protein, alix and heat shock protein (HSP)70/90 (18). Microvesicles, 100-1,000 nm in diameter, directly bud from the plasma membrane by outward blebbing, which is dependent on cytoskeletal rearrangements and calcium influx. The main molecular markers for microvesicles are annexin A1 and A2 (19). Apoptotic body, 500-4,000 nm in diameter, are released during apoptosis as fragmented portions of the dying cell. The main molecular markers for apoptotic bodies are annexin V, phosphatidylserine and caspases (20). Migrasomes, 0.5-3.0 μm in diameter, are a subtype of EVs that are released from the elongated membranous structure at the tail of cells during migration. First reported in 2015, they were named due to their close association with cell migration. The hallmark proteins of migrasomes are tetraspanin-4 and -7, which may help cells eliminate damaged organelles such as mitochondria, promoting the release of cellular contents (including protein, mRNA and miRNA) for absorption by recipient cells (21). Large oncosomes are atypically large EVs (1-10 μm in diameter) that arise from non-apoptotic membrane blebbing. This process can be triggered through silencing of diaphanous-related formin-3 and overexpression of oncoproteins such as protein kinase B, heparin binding-epidermal growth factor and caveolin-1 (22). Compared with the large EVs, the small ectosomes (≤ 100 nm) has a notably higher percentage of CD9- and CD81-positive particles (23). A summary of the detailed types and classifications of EVs is provided in Table I (18-23).

Separation of EVs. EVs are secreted by cells into their surrounding environment and exist as a variety of types, including exosomes, microvesicles and apoptotic bodies. As different types of EVs perform distinct biological functions, their study is inherently dependent on the isolation and purification of specific EVs subpopulations. This section summarizes the principles, advantages and disadvantages of various EVs isolation methods (Table II) (24-35).

The choice of isolation and purification methods directly determines the yield, purity and physicochemical properties of the obtained EVs. Consequently, selecting a method that is efficient, convenient and reliable based on the characteristics

Table I. Classification of EVs.

Type	Category of EVs	Size, nm	Biogenesis	Molecular markers	(Refs.)
Exosomes	Small	30-150	Multivesicular endosomes	Tetraspanin protein family: CD63, CD81 and CD9; ESCRT-related proteins: Alix and TSG101; HSPs: HSP70 and HSP90	(18)
Microvesicles	Medium/large	100-1,000	Ectosomes	Annexin A1 and A2, and α -actinin 4	(19)
Apoptotic bodies	Large	500-5,000	Apoptosis	Annexin V, phosphatidylserine, caspases	(20)
Migrasomes	Large	500-3,000	Retraction fibers	TSPAN4 and TSPAN7	(21)
Large oncosomes	Large	1,000-10,000	Ectosomes	ARF6, V-ATPase G1, CK18 and annexin A1	(22)
Small ectosomes	Small	30-150	Ectosomes	CD147 and CD9	(23)

All EV classes possess a lipid bilayer membrane. Alix, ALG-2-interacting protein; ARF6, ADP-ribosylation factor 6; CK18, cytokeratin 18; ESCRT, endosomal sorting complex required for transport; EVs, extracellular vesicles; HSP, heat shock protein; TSG101, tumor susceptibility gene 101; TSPAN4, tetraspanin-4; TSPAN7, tetraspanin-7; V-ATPase, vacuolar-type ATPase.

Table II. EVs separation methods.

Separation method	Advantages	Disadvantages	(Refs.)
According to EV size			
Ultracentrifugation	Density gradient differences	Induce EV aggregation and morphological alterations, potentially modifying their composition and phenotype	(24,25)
Size exclusion chromatography	Suitable for removing protein aggregates and lipoprotein particles	Limited recovery rate, typically reducing the total particle number by approximately half	(26)
High-performance liquid chromatography	Maintains the integrity and biological activity of exosomes; high sample purity with low co-precipitation	Limited resolution and sample capacity, and inability to completely remove specific contaminants	(27)
Protein organic solvent precipitation	Novel and inexpensive method of rapidly isolating EVs from small volumes of human blood plasma	Reduces cell viability <i>in vitro</i>	(28)
According to EVs surface protein markers			
Tangential flow filtration	High-throughput filtration method for the reliable and specific separation of exosomes in biological fluids	Isolated EVs may be contaminated with proteins and lipid droplets; often requires additional purification steps to achieve high purity	(29,30)
Tangential flow for analyte capture	Novel method for isolating micro- and nano-scale species	Membrane adsorption and sample loss; inherent size-based limitations and restricted resolution	(31)
Heparin sulfate proteoglycan	Enhances the purification efficiency of EVs with lower contamination levels	Some proteins in media and biofluids can bind heparin	(32)

Table II. Continued.

Separation method	Advantages	Disadvantages	(Refs.)
According to other features			
Microfluidic technique	Standardized and rapid method that can be adjusted for EVs from different cell sources	Limited clinical applicability due to low isolation throughput	(33)
Flow cytometry	Primary technique for EV analysis and detection of specific subtypes	Limited fluorescence sensitivity	(34)
Polymer precipitation method	Simple, robust and cost-effective method; successfully yields large quantities of EVs from natural killer cells	Non-specific co-precipitation	(35)

EV, extracellular vesicle.

of the target EVs is essential. However, common isolation methods often fail to completely eliminate soluble contaminants and non-vesicular particles from cell culture supernatants or biological fluids. Therefore, it is necessary to develop novel isolation strategies that offer high specificity, purity and scalability for large-scale production. Established methods for EVs isolation can be broadly categorized into several categories.

Separation according to size. Ultracentrifugation remains the predominant method for EV isolation, enabling the separation of vesicles based on size and density, often through sucrose or iodixanol density gradients (36). However, this technique can introduce undesirable artifacts, including EV aggregation and morphological alterations, which may affect vesicular integrity, composition and biological activity (24,37). A further limitation is that ultracentrifugation alone may not fully eliminate contamination from non-vesicular components. To mitigate these issues, methods combining ultracentrifugation with other techniques have been developed. For example, combining ultracentrifugation with cryo-electron microscopy and immunogold labeling has been shown to preserve EVs diversity and prevent aggregation (38). Similarly, ultracentrifugation can be combined with filtration techniques, including tangential flow filtration (TFF) and microfluidic filtration, to reduce contaminants and enhance separation efficiency (39,40).

Several complementary methods are available for EV isolation, including density gradient centrifugation, size exclusion chromatography (SEC), high-performance liquid chromatography and integrated or combination strategies. SEC is particularly effective at removing protein aggregates and lipoprotein particles; however, its recovery rate is limited, typically reducing the total particle number by approximately half (41,42). Furthermore, SEC is unsuitable for initial volume reduction during EV extraction, such as in the isolation of EVs from cell culture supernatants (43). In addition, TFF is a high-throughput method for the efficient and selective

separation of EVs from biological fluids. It has been used as an early purification step following the removal of cellular debris via low-speed centrifugation and filtration through 0.22- μ m membranes (44). Another promising method for the rapid isolation of EVs is protein organic solvent precipitation, which effectively removes soluble protein contaminants and yields EVs of higher purity compared with TFF, demonstrating potential for clinical translation (45).

Separation according to surface protein markers. The heterogeneity of EVs encompasses subpopulations characterized by specific surface markers, enabling their selective isolation. For example, HSPs commonly found on EVs derived from cancerous or infected cells enable targeted capture. Virucine peptides have been shown to exhibit high efficiency in the isolation of HSP-bearing EVs from diverse sources, including cell culture media, plasma and urine (46). Heparan sulfate proteoglycan, a cell surface receptor involved in various biological processes, can also be used to enhance EVs purification. For example, when conditioned media from 293T cells was mixed with heparin-coated agarose beads following ultracentrifugation, an EVs recovery rate of 60% was achieved (47). This method also reduces contamination while preserving EVs-associated proteins and other biomarkers (48).

TFF operates on the principle of parallel fluid flow across a membrane to reduce clogging. This technique is superior to traditional ultracentrifugation as it effectively maintains both high separation efficiency and the biological integrity of EVs. Tangential flow for analyte capture (TFAC) is a novel method built upon TFF. It enables the selective capture of target exosomes using functionalized membranes modified with specific antibodies, integrating both the capture and elution steps into a single process. This streamlined process minimizes impurities, enables the rapid processing of small clinical samples and yields high-purity exosomes. Due to its efficiency, scalability and preservation of EV bioactivity, TFAC is emerging as a promising method for EVs isolation (31).

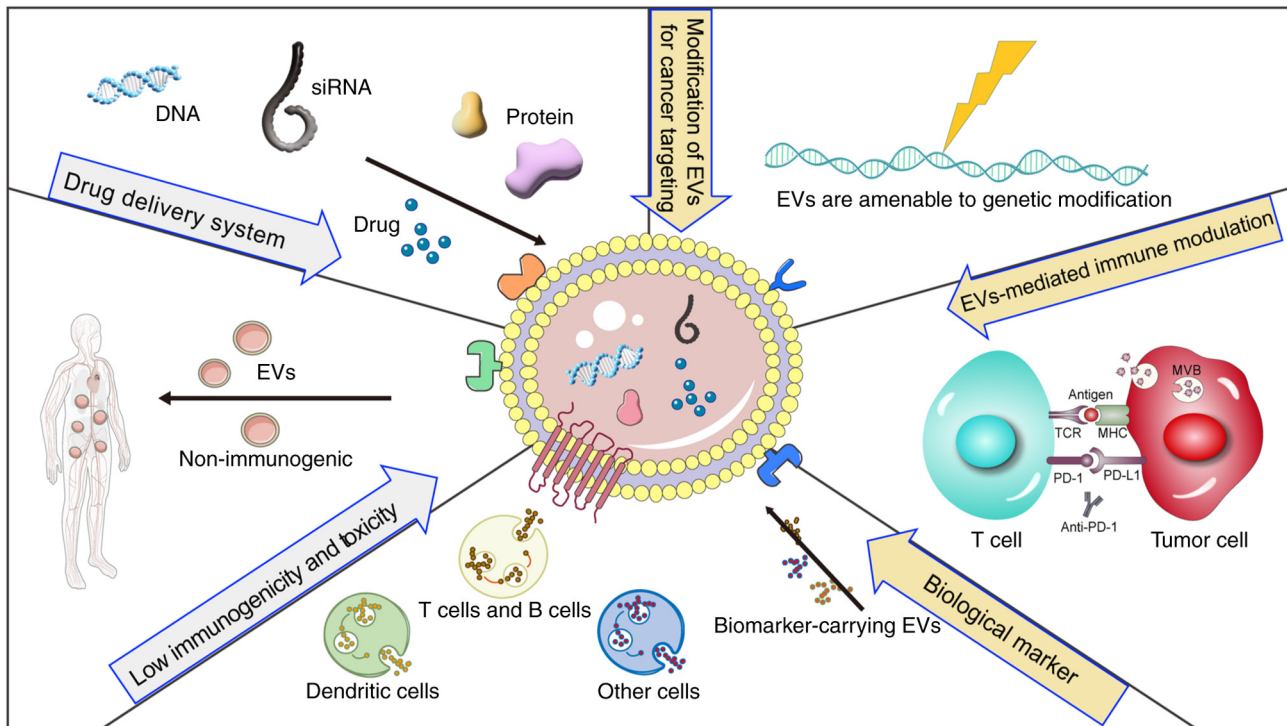


Figure 1. Schematic overview of the advantages and therapeutic potential of EVs. Certain nucleic acids and proteins are transported by EVs, which can be used as biomarkers for disease diagnosis. The targeting specificity of EVs toward particular cell types can be enhanced through genetic engineering or click-chemistry approaches. Also, EVs present multiple antigens that can induce immune tolerance, positioning them as a novel therapeutic strategy. EVs, extracellular vesicles; MHC, major histocompatibility complex; MVB, multivesicular body; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand 1; siRNA, small interfering RNA; TCR, T cell receptor.

These advancements address challenges in the mass production of EVs for clinical translation and help the transition from qualitative research to translational applications, thereby opening new avenues for regenerative medicine, drug delivery and liquid biopsy (44).

Separation according to other features. Flow cytometry is a key technique for analyzing and separating EVs. Following initial isolation by methods such as ultracentrifugation, it enables the quantification of EVs from various sources using fluorescent markers such as carboxyfluorescein succinimidyl ester and lipid-specific dyes. This technique is widely used to identify and characterize specific EVs subtypes. It employs antibody-fluorophore conjugates that target specific membrane antigens, allowing the detection and classification of individual particles based on their fluorescence characteristics, thereby enabling immunophenotyping and subgroup classification (41,49).

Immunological methods provide an alternative strategy for the isolation of EVs, particularly those derived from antigen-presenting cells (APCs), which release immunomodulatory EVs characterized by high levels of major histocompatibility complex II (MHCII) expression. Using immunomagnetic beads, exosomes can be efficiently enriched from cell-free supernatants, thereby accelerating the analysis of APC-derived EVs (42). In addition, microfluidic technology has been developed to capture CD41-positive exosomes on mica-coated surfaces using specific antibodies. Although this method allows the rapid and standardized isolation of EVs from various cell types, its clinical application is limited by its small-scale processing capacity. Furthermore, given

the overlapping physical properties of EVs and viruses, a CD45-based immunodepletion approach has been developed to specifically remove HIV particles without affecting CD45-positive EVs (50). Finally, polymer precipitation represents a cost-effective and robust technique for large-scale EV isolation. Although its use in clinical settings is limited, it has been successfully applied to obtain substantial quantities of functional EVs from natural killer (NK) cells *in vitro* (51).

Advantage of EVs in cancer research. The type and quantity of EVs reflect the physiological and pathological states. A large body of research has focused on exploring the potential benefits and therapeutic applications of EVs in malignant tumors (Fig. 1).

Low immunogenicity and toxicity. EVs offer key advantages over alternative delivery systems due to their low immunogenicity and minimal cytotoxicity. As EVs can be isolated from endogenous cellular sources, they trigger negligible immune responses, making them highly suitable for drug and gene delivery in tissue repair and regenerative medicine (44). The encapsulation of therapeutic agents within EVs not only reduces systemic toxicity but also enhances bioavailability by minimizing the immune-mediated clearance of the cargo prior to it reaching target tissues. For example, in a mouse model of pancreatic cancer, small interfering RNA (siRNA) delivered via exosomes demonstrated greater efficacy and fewer side effects than was achieved using NP-based delivery systems (52). By contrast, numerous nanomedicines undergo rapid immune clearance, which limits their clinical

effectiveness. Furthermore, the low immunogenicity and cytotoxicity of EVs enable large-scale use without typical toxic side effects. Studies have shown that artificial EVs injected into mice do not cause adverse reactions, highlighting their potential as therapeutic tools and providing a strong foundation for the clinical application of EV-based therapies (45).

Engineering EVs as potential drug delivery vehicles. EVs outperform liposomes in terms of drug delivery efficiency while maintaining low immunogenicity and toxicity. Although liposomes share a similar lipid bilayer structure with EVs and have been used as drug carriers for decades, they predominantly accumulate in liver and spleen macrophages, resulting in rapid clearance and reduced therapeutic efficacy. By contrast, EVs possess endogenous surface proteins that help them to evade immune detection, thereby extending their circulation time (53). For example, studies have shown that EVs loaded with porphyrins via electroporation, saponin treatment or dialysis exhibit higher loading efficiency compared with that of liposomes, highlighting the advantages of EVs as superior drug delivery carriers (54).

As aforementioned, EVs are membranous particles secreted by living cells into the extracellular space, where they play a key role in intercellular communication. Owing to their stability, broad tissue distribution and ability to cross biological barriers, EVs are emerging as promising drug delivery vehicles. They can encapsulate a diverse range of biological molecules, including microRNAs (miRNAs/miRs), siRNAs and recombinant proteins, that are otherwise difficult to deliver intracellularly without a carrier. Engineered EVs provide a targeted solution for the delivery of such molecules to specific sites (55,56).

Two primary strategies are used to direct EVs to cancer cells and enhance their accumulation at the tumor: i) Engineering the parent cells prior to EVs isolation and ii) directly modifying the membrane of purified EVs. Both approaches aim to improve the targeting specificity and efficacy of EVs-based delivery. For example, a fusion protein composed of apoptin and the EVs membrane protein CD9 was expressed in parent cells using light-responsive cleavable peptide linkers. This fusion protein was encapsulated within EVs, enabling the light-triggered release of apoptin (57). In another example, adipose-derived mesenchymal stem cells were transfected with a miR-122 plasmid to produce miR-122-enriched EVs. Intratumoral injection of these EVs increased the sensitivity of liver cancer cells to sorafenib (58).

As lipid-bound nanostructures, EVs naturally incorporate transmembrane proteins and surface glycans, including glycolipids. These native components act as natural anchoring sites for functionalizing EVs via genetic engineering or bioconjugation techniques. A wide range of bioactive molecules, including fluorescent probes, targeting peptides, therapeutic drugs, nanobodies and aptamers, can be displayed at these sites. For example, the GE11 peptide, which binds both epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor, was successfully expressed on the surface of engineered EVs derived from 293 cells, enabling targeted delivery to breast cancer cells with high levels of EGFR expression (59). In another approach, glycosylation, a common post-translational modification, was incorporated into a lysosome-associated membrane protein 2b-targeted

peptide fusion protein. This modification improved the stability of the peptide and enhanced its expression in both parent cells and EVs, thereby increasing targeting efficiency toward neuroblastoma cells (60).

EVs in tumor immunotherapy. EVs are released by nearly all cell types and play a crucial role in disease progression and pathogenesis by transporting signaling molecules, including proteins, lipids, mRNAs, miRNAs and other bioactive substances. As they can be sampled by minimally invasive procedures, EVs show considerable potential as clinically useful biomarkers (61). Tumor development is influenced by dynamic interactions between tumor cells and immune cells within the TME. Tumor cell-derived EVs (TDEVs) contribute to the modulation of immune responses by transferring macromolecules to recipient cells, thereby acting as carriers of immune signals and contributing to immune surveillance (62). For example, TDEVs can promote immune evasion by suppressing the co-stimulation of T cells and dendritic cells (DCs), thereby conferring resistance to immune checkpoint inhibitor (ICI) therapies (63). In hepatocellular carcinoma, EVs secreted by hepatocytes deficient in the gluconeogenic enzyme fructose-1,6-bisphosphatase 1 have been shown to target infiltrating NK cells, resulting in their dysfunction and depletion, which facilitates immune escape and tumor progression through remodeling of the immune microenvironment (64,65).

Conversely, EVs can also support antitumor immunity. NK cell-derived exosomes can deliver cytotoxic agents such as perforin and granzymes, which directly induce the lysis of melanoma cells (66). In addition, EVs derived from MHCII-expressing DCs can induce antigen-specific CD4⁺ T-cell activation (67). Furthermore, it has been reported that bone marrow DC-derived EVs regulate allograft rejection *in vivo*, and the intravenous administration of donor DC-derived EVs has been shown to delay acute rejection in rats receiving a heart transplant (68).

Exosomes are nanocapsules enriched in MHCII molecules and secreted by B-lymphoblast-like cells. They play a crucial role in intercellular communication, particularly in processes such as tumor angiogenesis and cell differentiation. Advances in exosome production and purification have enhanced their potential as versatile platforms for drug delivery, antigen presentation and biologically targeted therapy. Consequently, immune cell-derived exosomes are now widely regarded as efficient and natural nanocarriers capable of trafficking to target cells and modulating immune responses within the TME (69). The application of immune cell-derived exosomes in immunotherapy and vaccine development has been explored under both physiological and pathological conditions (70). ICI therapy is a well-established approach in tumor immunotherapy, which significantly increases life expectancy in some patients. However, only a minority of patients achieve clinical benefit, highlighting the need for reliable predictive biomarkers (71). Programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) inhibitors function by restoring the ability of suppressed T cells to recognize and kill tumor cells (71). Given the crucial role of EVs in tumor immune regulation, a combination of EVs and PD-L1 has been proposed as an effective biomarker for predicting patient response to ICI therapy. Notably, a clear dissociation has been observed between tissue-based and EV-based PD-L1

detection: while PD-L1 in tissue shows no association with survival, PD-L1 expression on circulating EVs reliably predicts survival in patients with non-small cell lung cancer (NSCLC). This dynamic, EV-based measurement offers a superior predictive model for the identification of patients who are likely to respond to ICI treatment and experience a survival benefit (72). In a study of 71 patients with metastatic melanoma (MM), circulating PD-L1⁺ and PD-1⁺ EVs were associated with responsiveness to ICI therapy. Circulating PD-1⁺ EVs, in particular, were implicated as a driving factor for resistance to anti-PD-1 therapy, suggesting a role in the stratification in patients with MM (73). These findings suggest that a comprehensive understanding of the biological roles of EVs within the TME is essential for effective cancer treatment and the development of effective cancer immunotherapies. Furthermore, the inhibition of EVs secretion pathways is currently being investigated as a therapeutic strategy (74). Combining targeted therapy that disrupt EV signaling with anti-PD-L1 therapy may potentially be more effective than anti-PD-L1 monotherapy

3. 3D cell cultures as prospective models to study EVs in cancer

Potential advantages of 3D cultivation over traditional 2D models. Historically, 2D cultures of primary cells and tumor cell lines have been widely used in cancer research and have provided valuable insights into tumor development and therapeutic mechanisms. However, 2D culture systems have inherent limitations, including an inability to recapitulate the genetic and phenotypic heterogeneity of tumors or accurately mimic the complex *in vivo* microenvironment (75). To more accurately model human tumor biology, 3D organoid culture systems have been developed. Organoids are self-organizing spheroidal structures derived from cancer stem cells or organ-specific progenitors with a 3D extracellular matrix. They exhibit self-renewal and differentiation capacity, while maintaining genetic and phenotypic stability over extended culture periods (76). By more closely recapitulating the structure and functional characteristics of primary tumor tissues, organoids have emerged as powerful tools in both basic and clinical cancer research. Their accessibility and experimental versatility make them particularly valuable for studying tumor immunity and mechanisms of drug resistance.

The limited predictive power of traditional *in vitro* models presents a major obstacle to the development of effective cancer immunotherapies. However, advances in 3D organoid-immune cell co-culture systems have enabled the establishment of immunocompetent tumor models that more accurately recapitulate the patient-specific TME. These models have transformed preclinical evaluation by enabling the functional screening of both immunotherapeutic and chemotherapeutic agents under conditions that mimic *in vivo* biology. Their application is exemplified by a study of prostate cancer in which the co-culture of organoids with cancer-associated fibroblasts (CAFs) demonstrated that CAF-derived neuregulin 1 promotes resistance to androgen therapy via HER3 activation in tumor cells (77). Similarly, Sebrell *et al* (78) showed that co-culturing monocyte-derived DCs with gastric cancer organoids enables patient-derived organoids (PDOs) to recruit

chemokines and DCs, thereby participating in immune surveillance during *Helicobacter pylori* infection. In another study, Dijkstra *et al* (79) demonstrate that co-cultures of autologous tumor organoids and peripheral blood lymphocytes can be used to enrich tumor-reactive T cells from the peripheral blood of patients with mismatch repair-deficient colorectal cancer and NSCLC. This may provide a basis for individualized treatment of T cells in patients with cancer. Collectively, these advances in organoid-immune cell co-culture technology are crucial for advancing tumor immunotherapy, enabling more precise investigation of tumor-immune interactions and offering valuable insights to inform clinical treatment strategies.

Applications of 3D model cultures in EV generation. Colorectal cancer organoids have been demonstrated to secrete significantly higher quantities of EVs than their conventional 2D-cultured counterparts (80). In colorectal cancer organoids, APC mutations that activate the Wnt pathway further promote EVs secretion in Matrigel-based cultures, a process that has been suggested to be facilitated by collagen, a key extracellular matrix component in matrigel (81). Another proposed mechanism involves the upregulation of specific transporters. For example, the ATP-binding cassette transporter G1 (ABCG1), a cholesterol efflux pump, is highly expressed in colon adenocarcinoma tumoroids with pronounced stemness features. Silencing ABCG1 inhibits EV release and results in the intracellular accumulation of vesicles (82).

Furthermore, the 3D architecture of organoids supports improved cell polarization and spatial asymmetry. Organoids derived from the LIM1863 colon carcinoma line have been shown to secrete two spatially distinct EV subtypes. Apically released EVs carry epithelial cell adhesion molecule (EPCAM) and are uniquely enriched in CD63, mucin 13, sucrase-isomaltase, dipeptidyl peptidase IV and prominin 1. By contrast, basolaterally released EVs contain the A33 glycoprotein and are associated with early endosome antigen 1, ADP-ribosylation factor and clathrin. These findings demonstrate that EPCAM-positive and A33-positive EVs are selectively sorted and secreted from the apical and basolateral membranes, respectively (83). This spatial organization indicates that distinct EV populations carrying different markers and cargo proteins are released from the apical and basal sides of cells in 3D culture (84).

Current challenges of combining EVs with organoid models in the TME. EVs mediate crucial intercellular communication within the TME by facilitating autocrine and paracrine signaling between tumor cells and stromal components (85). Elevated EV levels have been observed in numerous malignancies, where they transport molecular cargo essential for tumor progression, and serve as potential diagnostic and prognostic biomarkers (86). Also, in tumor-stromal interactions, EVs coordinate complex intercellular crosstalk, which has led to exploration of their applications in drug delivery and targeted therapies (87).

Organoids, through their 3D structure, recapitulate the structural and physiological complexity of *in vivo* tissues more accurately than do 2D monolayer cultures, and have emerged as powerful systems for tumor biology, regenerative medicine and EV research (75). The development of organoid technology

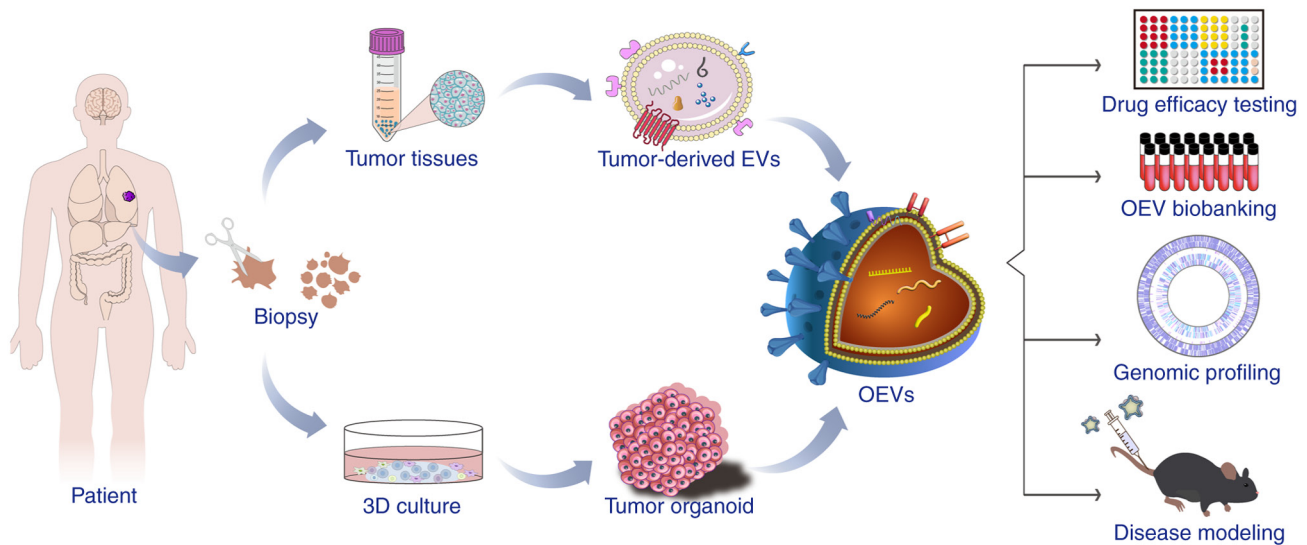


Figure 2. Synthesis and applications of OEVs. Tumor tissue samples are processed to obtain tissue-derived EVs and to generate organoids via 3D culture. The EVs transport bioactive molecules, including nucleic acids, lipids and proteins, to target cells. The combination of organoids and EVs yields OEVs, which have applications in diverse areas, including drug screening, biobanking, personalized medicine, genomic profiling and animal research. 3D, 3 dimensional; EVs, extracellular vesicles; OEVs, organoid-derived EVs.

has enabled the functional evaluation of EVs in physiologically relevant 3D models, offers alternative sources for EV production and the potential for advancing our understanding of human diseases (88).

Several studies illustrate the promise of this approach. For example, in an *in vitro* aging model, mouse intrahepatic bile duct-derived organoids co-cultured with exosomes from human placental mesenchymal stem cells demonstrated the ability to delay cellular senescence (89). In another study, Taha *et al* (90) revealed that matrix metalloproteinase 3 (MMP3) regulates tumor growth and EV integrity; MMP3 knockout disrupted EV structure and function, reduced tumor organoid proliferation, and suppressed tumor progression, highlighting the protumorigenic role of MMP3 in the maintenance of organoid and EV integrity. In addition, the similarity of small RNA profiles of EVs from 3D cervical cancer spheroids to *in vivo*-derived EVs is greater than that of 2D cultures, underscoring the physiological relevance of 3D models in EV research (91).

Multiple studies have demonstrated that EVs loaded with specific miRNAs can regulate immune responses, chemotherapy resistance and metastatic processes in various cancers. For example, esophageal adenocarcinoma (EAC)-derived EVs promote the formation of multilayered gastric organoids through the delivery of miR-25 and miR-210, establishing a novel co-culture model of EAC-EVs with 3D organoids (92). Similarly, ovarian cancer-derived EVs from malignant ascites and plasma enhance tumor aggressiveness and organoid growth via the transfer of miR-1246 and miR-1290 (93). In addition, in a study of organoids derived from patients with pancreatic ductal adenocarcinoma, the EVs miRNA profiles of the organoids revealed significantly upregulated levels of miR-21 and miR-195, matching those in patient plasma. This further validates tumor-derived organoids as physiologically relevant models for the analysis of EVs cargo (94).

Collectively, these findings indicate that tumor-derived organoids serve as robust platform for analyzing the molecular cargo of tumor cell-derived EVs. Emerging strategies integrating EVs with organoid models are enhancing our ability to recapitulate and evaluate dynamic disease processes. EV-loaded organoid systems provide synergistic advantages for simulating complex tumor-matrix interactions within the TME, and studies have leveraged EV-organoid co-culture platforms to elucidate TME-mediated mechanisms of tumor progression and therapeutic resistance (95,96).

Overall, tumor organoids are a valuable platform for studying EVs biology, while EVs enhance the physiological relevance of organoids in cancer research. Consequently, the integration of organoid and EVs technologies to generate organoid-derived EVs (OEVs) holds considerable promise across multiple research domains (Fig. 2).

4. Current progress and prospects of EVs-organoid therapies

Tumor-derived EVs are closely associated with key oncogenic processes, including drug resistance, TME remodeling, angiogenesis and distant metastasis (83). However, EVs obtained from conventional cell cultures may not fully capture the dynamic changes that occur under pathological conditions. EVs isolated from bodily fluids such as blood, urine, saliva and cerebrospinal fluid can more accurately reflect disease-related alterations. Despite this advantage, they only partially represent tumor-specific changes and are subject to inter-sample variability. These limitations can be addressed by isolating EVs directly from tissue samples. Compared with body fluid-derived EVs, EVs isolated directly from tumor tissues contain fewer contaminants and originate from a more defined cellular context. They also enable the study of specific cellular subpopulations at tumor sites, offering a more precise representation of tumor-TME interactions (97). Tissue-derived EVs

also enable the characterization of physiologically relevant EVs subsets enriched within the tumor site (98). For example, one study reported the isolation of EVs from frozen biopsy samples of primary and metastatic melanoma, achieving high purity, sensitivity and reproducibility. Genomic and proteomic analyses of these samples were performed to investigate the mechanisms by which patient-derived organoid (PDO)-derived EVs regulate the TME (99). In another study, breast cancer tissue-derived EVs were shown to retain genetic characteristics of the original tumor and were capable of promoting diabetic wound healing by suppressing oxidative stress (100).

The development of organoids is based on stem cell biology and developmental biology principles, mimicking natural developmental processes to generate miniature organ structures composed of lineage-specific cell types. They replicate the spatial architecture and physiological functions of human tissues and organs, providing a highly relevant system for modeling both normal physiology and pathology. As next-generation *in vitro* biological models, organoids have emerged as a dynamic and innovative platform in biomedical research (101).

Despite their considerable potential, current organoid culture systems face several challenges that limit their widespread application. One major limitation is the general absence of vascularization in most organoids. As organoids increase in size, they become constrained by inadequate oxygen supply and the accumulation of metabolic waste, which can lead to central necrosis (102). To address this issue, attempts have been made to construct tumor organoids within a microenvironment comprising vascular endothelial cells or by co-culturing organoid tumor cells with vascular endothelial cells to promote the generation of vascular structures (103). Another limitation is that individual organoid models primarily assess drug effects on a single target tissue without predicting potential side effects in other organ systems. This underscores the requirement for comprehensive organoid biobanks that enable the systematic evaluation of drug efficacy and potential toxicity across multiple tissue types (104).

Integrating multiple disciplines and model systems is essential for advancing the study of disease progression. For example, 3D bioprinting is an emerging manufacturing technology that offers the potential for constructing heterogeneous cellular microenvironments. This may accelerate organoid research by enabling precise, scalable and reproducible fabrication of 3D cellular structures. Therefore, the combination of organoids and 3D bioprinting technology may facilitate the development of functional therapies based on organoid technology (105). Zhang *et al* (98) summarized the biogenesis, structure and isolation mechanisms of OEVs. Compared with conventional EVs, OEVs can be produced in higher yields and exhibit superior physiological relevance. Organoids possess stem cell characteristics, and OEVs are capable of delivering active substances, suggesting that both have potential medical applications. They can also be engineered to enhance their therapeutic and targeting properties, showing promise for the treatment of diseases including inflammatory bowel disease, cancer, retinal disorders and brain diseases. However, substantial challenges remain. These include the development of scaled up OEV production methods to meet clinical demands, the elucidation of the mechanisms by which OEVs promote tissue repair, and establishment of standardized guidelines for their clinical application.

5. Conclusions and future perspectives

Intercellular communication is a fundamental mechanism in cancer progression and metastasis. As key mediators of cell-cell signaling within the TME, EVs regulate cellular homeostasis, tumor development, immune regulation and other pathological processes through their unique mode of cargo-based information transfer. Growing evidence indicates that EVs offer substantial advantages over traditional synthetic carriers, and have important applications as biomarkers and drug delivery systems in disease diagnosis and treatment.

Conventional 2D cell cultures fail to simulate the complex cell-cell and cell-matrix interactions characteristic of the native TME, limiting their translational relevance. By contrast, organoids as 3D tissue analogs closely mimic the structure and function of *in vivo* tissues, providing a more physiologically representative experimental model. The development of patient-derived 3D organoid cultures represents a major advancement, as these self-organizing structures faithfully replicate the architectural, genetic and phenotypic heterogeneity of primary tumors. The use of organoids to investigate specific EVs functions has broad application potential in regenerative medicine and oncology. The strategic integration of EV biology with 3D organoid technology establishes a novel and powerful research paradigm. Supplementing organoid cultures with tumor-derived EVs, or co-culturing tumor organoids with stromal cell-derived EVs, creates more physiologically relevant models that capture the paracrine and endocrine signaling networks active in cancer.

The present review has summarized the value of organoids as promising models for studying the TME and tumor immunity, examined research progress in the combination of organoid and EVs approaches in malignancies, and highlighted emerging applications and challenges associated with OEVs. However, OEV research remains at an early stage. Further efforts are necessary to deepen mechanistic understanding and optimize culture and isolation methods, thereby supporting their clinical translation and practical application.

Acknowledgements

Not applicable.

Funding

This study was supported by Ningxia Autonomous Region Key R&D Programs (grant no. 2022BEG03124), Ningxia Natural Science Foundation (grant no. 2025AAC030763), Ningxia Natural Science Foundation (grant no. 2024AAC03587) and Special Talent Introduction Project of Ningxia Autonomous Region Key R&D Programs (grant no. 2023BSB03054).

Availability of data and materials

Not applicable.

Authors' contributions

JC and YX prepared the original draft and wrote the main content. YG created the figures and participated in writing the

draft. XL and LW supervised the manuscript revision process and revised the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Wessler S, Aberger F and Hartmann TN: The sound of tumor cell-microenvironment communication-composed by the cancer cluster salzburg research network. *Cell Commun Signal* 15: 20, 2017.
- Urabe F, Kosaka N, Ito K, Kimura T, Egawa S and Ochiya T: Extracellular vesicles as biomarkers and therapeutic targets for cancer. *Am J Physiol Cell Physiol* 318: C29-C39, 2020.
- Bebelman MP, Smit MJ, Pegtel DM and Baglio SR: Biogenesis and function of extracellular vesicles in cancer. *Pharmacol Ther* 188: 1-11, 2018.
- Ortiz A: Extracellular vesicles in cancer progression. *Semin Cancer Biol* 76: 139-142, 2021.
- Khan NLA, Muhandiram S, Dissanayake K, Godakumara K, Midekessa G, Andronowska A, Heath PR, Kodithuwakku S, Hart AR and Fazeli A: Effect of 3D and 2D cell culture systems on trophoblast extracellular vesicle physico-chemical characteristics and potency. *Front Cell Dev Biol* 12: 1382552, 2024.
- Zhao H, Jiang E and Shang Z: 3D co-culture of cancer-associated fibroblast with oral cancer organoids. *J Dent Res* 100: 201-208, 2021.
- Campora S and Lo Cicero A: The 3D language of cancer: Communication via extracellular vesicles from tumor spheroids and organoids. *Int J Mol Sci* 26: 7104, 2025.
- Fiorini E, Veghini L and Corbo V: Modeling cell communication in cancer with organoids: Making the complex simple. *Front Cell Dev Biol* 8: 166, 2020.
- Chargaff E and West R: The biological significance of the thromboplastic protein of blood. *J Biol Chem* 166: 189-197, 1946.
- Anderson HC: Vesicles associated with calcification in the matrix of epiphyseal cartilage. *J Cell Biol* 41: 59-72, 1969.
- Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ and Geuze HJ: B lymphocytes secrete antigen-presenting vesicles. *J Exp Med* 183: 1161-1172, 1996.
- Izquierdo-Useros N, Puertas MC, Borràs FE, Blanco J and Martínez-Picado J: Exosomes and retroviruses: the chicken or the egg? *Cell Microbiol* 13: 10-17, 2011.
- Mittelbrunn M and Sánchez-Madrid F: Intercellular communication: Diverse structures for exchange of genetic information. *Nat Rev Mol Cell Biol* 13: 328-335, 2012.
- Abecassis MM, Burke R, Klintmalm GB, Matas AJ, Merion RM, Millman D, Olthoff K and Roberts JP; American Society of Transplant Surgeons: American society of transplant surgeons transplant center outcomes requirements-a threat to innovation. *Am J Transplant* 9: 1279-1286, 2009.
- Jeppesen DK, Zhang Q, Franklin JL and Coffey RJ: Extracellular vesicles and nanoparticles: Emerging complexities. *Trends Cell Biol* 33: 667-681, 2023.
- Raposo G and Stoorvogel W: Extracellular vesicles: Exosomes, microvesicles, and friends. *J Cell Biol* 200: 373-383, 2013.
- Xu R, Greening DW, Zhu HJ, Takahashi N and Simpson RJ: Extracellular vesicle isolation and characterization: Toward clinical application. *J Clin Invest* 126: 1152-1162, 2016.
- Krylova SV and Feng D: The machinery of exosomes: Biogenesis, release, and uptake. *Int J Mol Sci* 24: 1337, 2023.
- Menck K, Sivaloganathan S, Bleckmann A and Binder C: Microvesicles in cancer: Small size, large potential. *Int J Mol Sci* 21: 5373, 2020.
- Xu X, Lai Y and Hua ZC: Apoptosis and apoptotic body: Disease message and therapeutic target potentials. *Biosci Rep* 39: BSR20180992, 2019.
- Ma Y, Li T, Zhao L, Zhou D, Dong L, Xu Z, Wang Y, Yao X and Zhao K: Isolation and characterization of extracellular vesicle-like nanoparticles derived from migrasomes. *FEBS J* 290: 3359-3368, 2023.
- Minciacchi VR, You S, Spinelli C, Morley S, Zandian M, Aspuria PJ, Cavallini L, Ciardiello C, Reis Sobreiro M, Morello M, *et al*: Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles. *Oncotarget* 6: 11327-11341, 2015.
- Mathieu M, Névo N, Jouve M, Valenzuela JI, Maurin M, Verweij FJ, Palmulli R, Lankar D, Dingli F, Loew D, *et al*: Specificities of exosome versus small ectosome secretion revealed by live intracellular tracking and synchronized extracellular vesicle release of CD9 and CD63. *bioRxiv*, 2020.
- Théry C, Amigorena S, Raposo G and Clayton A: Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol Chapter* 3: Unit 3.22, 2006.
- Colombo M, Raposo G and Théry C: Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 30: 255-289, 2014.
- de Menezes-Neto A, Sáez MJF, Lozano-Ramos I, Segui-Barber J, Martín-Jaular L, Ullate JME, Fernández-Becerra C, Borràs FE and Del Portillo HA: Size-exclusion chromatography as a stand-alone methodology identifies novel markers in mass spectrometry analyses of plasma-derived vesicles from healthy individuals. *J Extracell Vesicles* 4: 27378, 2015.
- Huang T and He J: Characterization of extracellular vesicles by size-exclusion high-performance liquid chromatography (HPLC). In: *Extracellular Vesicles: Methods and Protocols*. Springer, New York, NY, pp191-199, 2017.
- Gallart-Palau X, Serra A, Wong ASW, Sandin S, Lai MKP, Chen CP, Kon OL and Sze SK: Extracellular vesicles are rapidly purified from human plasma by PReoteIn organic solvent PReecipitation (PROSPR). *Sci Rep* 5: 14664, 2015.
- Heinemann ML, Ilmer M, Silva LP, Hawke DH, Recio A, Vorontsova MA, Alt E and Vykoukal J: Benchtop isolation and characterization of functional exosomes by sequential filtration. *J Chromatogr A* 1371: 125-135, 2014.
- Böing AN, van der Pol E, Grootemaat AE, Coumans FA, Sturk A and Nieuwland R: Single-step isolation of extracellular vesicles by size-exclusion chromatography. *J Extracell Vesicles* 3: 10.3402/jev.v3.23430, 2014.
- Dehghani M, Lucas K, Flax J, McGrath J and Gaborski T: Tangential flow microfluidics for the capture and release of nanoparticles and extracellular vesicles on conventional and ultrathin membranes. *Adv Mater Technol* 4: 1900539, 2019.
- Li R, Wang C, Zhou M, Liu Y, Chen S, Chai Z, Huang H, Zhang K, Han Z, Hua G, *et al*: Heparan sulfate proteoglycan-mediated internalization of extracellular vesicles ameliorates liver fibrosis by targeting hepatic stellate cells. *Extracell Vesicle* 1: 100018, 2022.
- Zhu L, Du J, Cheng X, Hu R, Li X, Chen X and Xu S: Microfluidic innovations for enhanced extracellular vesicle isolation and analysis: A comprehensive review. *Anal Chem* 97: 4695-4705, 2025.
- Shen W, Guo K, Adkins GB, Jiang Q, Liu Y, Sedano S, Duan Y, Yan W, Wang SE, Bergersen K, *et al*: A single extracellular vesicle (EV) flow cytometry approach to reveal EV heterogeneity. *Angew Chem Int Ed Engl* 57: 15675-15680, 2018.
- Iannotta D, A, Lai A, Nair S, Koifman N, Lappas M, Salomon C and Wolfram J: Chemically-induced lipoprotein breakdown for improved extracellular vesicle purification. *Small* 20: e2307240, 2024.
- Gardiner C, Di Vizio D, Sahoo S, Théry C, Witwer KW, Wauben M and Hill AF: Techniques used for the isolation and characterization of extracellular vesicles: Results of a worldwide survey. *J Extracell Vesicles* 5: 32945, 2016.
- Issman L, Brenner B, Talmon Y and Aharon A: Cryogenic transmission electron microscopy nanostructural study of shed microparticles. *PLoS One* 8: e83680, 2013.
- Klymiuk MC, Balz N, Elashry MI, Heimann M, Wenisch S and Arnhold S: Exosomes isolation and identification from equine mesenchymal stem cells. *BMC Vet Res* 15: 42, 2019.

39. Davies RT, Kim J, Jang SC, Choi EJ, Gho YS and Park J: Microfluidic filtration system to isolate extracellular vesicles from blood. *Lab Chip* 12: 5202-5210, 2012.
40. Ghosh A, Davey M, Chute IC, Griffiths SG, Lewis S, Chacko S, Barnett D, Crapoulet N, Fournier S, Joy A, *et al*: Rapid isolation of extracellular vesicles from cell culture and biological fluids using a synthetic peptide with specific affinity for heat shock proteins. *PLoS One* 9: e110443, 2014.
41. Clayton A, Court J, Navabi H, Adams M, Mason MD, Hobot JA, Newman GR and Jasani B: Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry. *J Immunol Methods* 247: 163-174, 2001.
42. Sheikh M, Bagga H, Bhojwani Y and Telrandhe U: The role of 3D culture models and advanced chromatography in exosome research for triple-negative breast cancer. *J Egypt Natl Canc Inst* 37: 67, 2025.
43. Lamichhane TN, Sokic S, Schardt JS, Raiker RS, Lin JW and Jay SM: Emerging roles for extracellular vesicles in tissue engineering and regenerative medicine. *Tissue Eng Part B Rev* 21: 45-54, 2015.
44. Agrawal P, Wilkstein K, Guinn E, Mason C, Serrano Martinez CI and Saylae J: A review of tangential flow filtration: Process development and applications in the pharmaceutical industry. *Org Process Res Dev* 27: 571-591, 2023.
45. van der Meel R, Fens MHAM, Vader P, van Solinge WW, Eniola-Adefeso O and Schiffelers RM: Extracellular vesicles as drug delivery systems: Lessons from the liposome field. *J Control Release* 195: 72-85, 2014.
46. Saito RF, Machado CML, Lomba ALO, Otake AH and Rangel MC: Heat shock proteins mediate intercellular communications within the tumor microenvironment through extracellular vesicles. *Appl Biosci* 3: 45-58, 2024.
47. Balaj L, Atai NA, Chen W, Mu D, Tannous BA, Breakefield XO, Skog J and Maguire CA: Heparin affinity purification of extracellular vesicles. *Sci Rep* 5: 10266, 2015.
48. Cerezo-Magaña M, Bång-Rudenstam A and Belting M: The pleiotropic role of proteoglycans in extracellular vesicle mediated communication in the tumor microenvironment. *Semin Cancer Biol* 62: 99-107, 2020.
49. Navasiolava NM, Dignat-George F, Sabatier F, Larina IM, Demiot C, Fortrat JO, Gauquelin-Koch G, Kozlovskaya IB and Custaud MA: Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers. *Am J Physiol Heart Circ Physiol* 299: H248-H256, 2010.
50. Jödicke RA, Huo S, Kränkel N, Piper SK, Ebinger M, Landmesser U, Flöel A, Endres M and Nave AH: The Dynamic of extracellular vesicles in patients with subacute stroke: Results of the 'biomarkers and perfusion-training-induced changes after stroke' (BAPTISE) study. *Front Neurol* 12: 731013, 2021.
51. Jong AY, Wu CH, Li J, Sun J, Fabbri M, Wayne AS and Seeger RC: Large-scale isolation and cytotoxicity of extracellular vesicles derived from activated human natural killer cells. *J Extracell Vesicles* 6: 1294368, 2017.
52. Jang SC, Kim OY, Yoon CM, Choi DS, Roh TY, Park J, Nilsson J, Lötvall J, Kim YK and Gho YS: Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. *ACS Nano* 7: 7698-7710, 2013.
53. Buzas EI: The roles of extracellular vesicles in the immune system. *Nat Rev Immunol* 23: 236-250, 2023.
54. Wang L, Wang D, Ye Z and Xu J: Engineering extracellular vesicles as delivery systems in therapeutic applications. *Adv Sci (Weinh)* 10: e2300552, 2023.
55. Lou G, Song X, Yang F, Wu S, Wang J, Chen Z and Liu Y: Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. *J Hematol Oncol* 8: 122, 2015.
56. Zhao S, Di Y, Fan H, Xu C, Li H, Wang Y, Wang W, Li C and Wang J: Targeted delivery of extracellular vesicles: The mechanisms, techniques and therapeutic applications. *Mol Biomed* 5: 60, 2024.
57. Kowalczyk A, Gajda-Walczak A, Ruzycza-Ayoush M, Targonska A, Mosieniak G, Glogowski M, Szumera-Cieckiewicz A, Prochorec-Sobieszek M, Bamburowicz-Klimkowska M, Nowicka AM and Grudzinski IP: Parallel SPR and QCM-D quantitative analysis of CD9, CD63, and CD81 tetraspanins: A simple and sensitive way to determine the concentration of extracellular vesicles isolated from human lung cancer cells. *Anal Chem* 95: 9520-9530, 2023.
58. Cao M, Isaac R, Yan W, Ruan X, Jiang L, Wan Y, Wang J, Wang E, Caron C, Neben S, *et al*: Cancer-cell-secreted extracellular vesicles suppress insulin secretion through miR-122 to impair systemic glucose homeostasis and contribute to tumour growth. *Nat Cell Biol* 24: 954-967, 2022.
59. Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, Ohgi T, Ochiya T, *et al*: Systemically injected exosomes targeted to EGFR deliver anti-tumor microRNA to breast cancer cells. *Mol Ther* 21: 185-191, 2013.
60. Hung ME and Leonard JN: Stabilization of exosome-targeting peptides via engineered glycosylation. *J Biol Chem* 290: 8166-8172, 2015.
61. Wan M, Ning B, Spiegel S, Lyon CJ and Hu TY: Tumor-derived exosomes (TDEs): How to avoid the sting in the tail. *Med Res Rev* 40: 385-412, 2020.
62. Zhao X, Yuan C, Wangmo D and Subramanian S: Tumor-secreted extracellular vesicles regulate T-cell costimulation and can be manipulated to induce tumor-specific T-cell responses. *Gastroenterology* 161: 560-574.e11, 2021.
63. Rahmat JN, Liu J, Chen T, Li Z and Zhang Y: Engineered biological nanoparticles as nanotherapeutics for tumor immunomodulation. *Chem Soc Rev* 53: 5862-5903, 2024.
64. Liu Z, You Y, Chen Q, Li G, Pan W, Yang Q, Dong J, Wu Y, Bei JX, Pan C, *et al*: Extracellular vesicle-mediated communication between hepatocytes and natural killer cells promotes hepatocellular tumorigenesis. *Mol Ther* 30: 606-620, 2022.
65. Pizzuttilo EG, Romanò R, Roazzi L, Agostara AG, Oresti S, Zepellini A, Giannetta L, Cerea G, Signorelli D, Siena S and Sartore-Bianchi A: Immune checkpoint inhibitors and the exosome: Host-extrinsic factors determine response, survival, and toxicity. *Cancer Res* 83: 2283-2296, 2023.
66. Zhu L, Kalimuthu S, Gangadaran P, Oh JM, Lee HW, Baek SH, Jeong SY, Lee SW, Lee J and Ahn BC: Exosomes derived from natural killer cells exert therapeutic effect in melanoma. *Theranostics* 7: 2732, 2017.
67. Théry C, Duban L, Segura E, Véron P, Lantz O and Amigorena S: Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. *Nat Immunol* 3: 1156-1162, 2002.
68. Pêche H, Heslan M, Usal C, Amigorena S and Cuturi MC: Presentation of donor major histocompatibility complex antigens by bone marrow dendritic cell-derived exosomes modulates allograft rejection. *Transplantation* 76: 1503-1510, 2003.
69. Li Q, Wang H, Peng H, Huyan T and Cacalano NA: Exosomes: Versatile nano mediators of immune regulation. *Cancers (Basel)* 11: 1557, 2019.
70. Zhao Y, Liu T and Zhou M: Immune-cell-derived exosomes for cancer therapy. *Mol Pharm* 19: 3042-3056, 2022.
71. Yang F, Wang JF, Wang Y, Liu B and Molina JR: Comparative analysis of predictive biomarkers for PD-1/PD-L1 inhibitors in cancers: Developments and challenges. *Cancers (Basel)* 14: 109, 2021.
72. de Miguel-Perez D, Russo A, Arrieta O, Ak M, Barron F, Gunasekaran M, Mamindla P, Lara-Mejia L, Peterson CB, Er ME, *et al*: Extracellular vesicle PD-L1 dynamics predict durable response to immune-checkpoint inhibitors and survival in patients with non-small cell lung cancer. *J Exp Clin Cancer Res* 41: 186, 2022.
73. Serrati S, Guida M, Di Fonte R, De Summa S, Strippoli S, Iacobazzi RM, Quarta A, De Risi I, Guida G, Paradiso A, *et al*: Circulating extracellular vesicles expressing PD1 and PD-L1 predict response and mediate resistance to checkpoint inhibitors immunotherapy in metastatic melanoma. *Mol Cancer* 21: 20, 2022.
74. Abreu SC, Lopes-Pacheco M, Weiss DJ and Rocco PRM: Mesenchymal stromal cell-derived extracellular vesicles in lung diseases: Current status and perspectives. *Front Cell Dev Biol* 9: 600711, 2021.
75. Rauner G, Gupta PB and Kuperwasser C: From 2D to 3D and beyond: The evolution and impact of in vitro tumor models in cancer research. *Nat Methods* 22: 1776-1787, 2025.
76. Eiraku M and Sasai Y: Self-formation of layered neural structures in three-dimensional culture of ES cells. *Curr Opin Neurobiol* 22: 768-777, 2012.
77. Liu J, Li P, Wang L, Li M, Ge Z, Noordam L, Lieshout R, Verstegen MMA, Ma B, Su J, *et al*: Cancer-associated fibroblasts provide a stromal niche for liver cancer organoids that confers trophic effects and therapy resistance. *Cell Mol Gastroenterol Hepatol* 11: 407-431, 2021.
78. Sebrell TA, Hashimi M, Sidar B, Wilkinson RA, Kirpotina L, Quinn MT, Malkoç Z, Taylor PJ, Wilking JN and Bimczok D: A novel gastric spheroid co-culture model reveals chemokine-dependent recruitment of human dendritic cells to the gastric epithelium. *Cell Mol Gastroenterol Hepatol* 8: 157-171.e3, 2019.

79. Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, Fanchi LF, Slagter M, van der Velden DL, Kaing S, Kelderman S, *et al*: Generation of tumor-reactive T cells by co-culture of peripheral blood lymphocytes and tumor organoids. *Cell* 174: 1586-1598.e12, 2018.
80. Abbas ZN, Al-Saffar AZ, Jasim SM and Sulaiman GM: Comparative analysis between 2D and 3D colorectal cancer culture models for insights into cellular morphological and transcriptomic variations. *Sci Rep* 13: 18380, 2023.
81. Szvicsek Z, Oszvald Á, Szabó L, Sándor GO, Kelemen A, Soós AA, Pálóczi K, Harsányi L, Tölgyes T, Dede K, *et al*: Extracellular vesicle release from intestinal organoids is modulated by Apc mutation and other colorectal cancer progression factors. *Cell Mol Life Sci* 76: 2463-2476, 2019.
82. Namba Y, Sogawa C, Okusha Y, Kawai H, Itagaki M, Ono K, Murakami J, Aoyama E, Ohyama K, Asaumi JI, *et al*: Depletion of lipid efflux pump ABCG1 triggers the intracellular accumulation of extracellular vesicles and reduces aggregation and tumorigenesis of metastatic cancer cells. *Front Oncol* 8: 376, 2018.
83. Yang Q, Xu J, Gu J, Shi H, Zhang J, Zhang J, Chen ZS, Fang X, Zhu T and Zhang X: Extracellular vesicles in cancer drug resistance: Roles, mechanisms, and implications. *Adv Sci (Weinh)* 9: e2201609, 2022.
84. Tauro BJ, Greening DW, Mathias RA, Mathivanan S, Ji H and Simpson RJ: Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. *Mol Cell Proteomics* 12: 587-598, 2013.
85. Maia J, Caja S, Strano Moraes MC, Couto N and Costa-Silva B: Exosome-based cell-cell communication in the tumor microenvironment. *Front Cell Dev Biol* 6: 18, 2018.
86. LeBleu VS and Kalluri R: Exosomes as a multicomponent biomarker platform in cancer. *Trends Cancer* 6: 767-774, 2020.
87. Jurj A, Zanoaga O, Braicu C, Lazar V, Tomuleasa C, Irimie A and Berindan-Neagoe I: A comprehensive picture of extracellular vesicles and their contents. *Molecular transfer to cancer cells. Cancers (Basel)* 12: 298, 2020.
88. Bordanaba-Florit G, Madarieta I, Olalde B, Falcón-Pérez JM and Royo F: 3D cell cultures as prospective models to study extracellular vesicles in cancer. *Cancers (Basel)* 13: 307, 2021.
89. Hwang WL, Lan HY, Cheng WC, Huang SC and Yang MH: Tumor stem-like cell-derived exosomal RNAs prime neutrophils for facilitating tumorigenesis of colon cancer. *J Hematol Oncol* 12: 10, 2019.
90. Taha EA, Sogawa C, Okusha Y, Kawai H, Oo MW, Elseoudi A, Lu Y, Nagatsuka H, Kubota S, Satoh A, *et al*: Knockout of MMP3 weakens solid tumor organoids and cancer extracellular vesicles. *Cancers (Basel)* 12: 1260, 2020.
91. Thippabhotla S, Zhong C and He M: 3D cell culture stimulates the secretion of in vivo like extracellular vesicles. *Sci Rep* 9: 13012, 2019.
92. Ke X, Yan R, Sun Z, Cheng Y, Meltzer A, Lu N, Shu X, Wang Z, Huang B, Liu X, *et al*: Esophageal adenocarcinoma-derived extracellular vesicle microRNAs induce a neoplastic phenotype in gastric organoids. *Neoplasia* 19: 941-949, 2017.
93. Wang W, Jo H, Park S, Kim H, Kim SI, Han Y, Lee J, Seol A, Kim J, Lee M, *et al*: Integrated analysis of ascites and plasma extracellular vesicles identifies a miRNA-based diagnostic signature in ovarian cancer. *Cancer Lett* 542: 215735, 2022.
94. Zeöld A, Sándor GO, Kiss A, Soós AA, Tölgyes T, Bursics A, Szűcs Á, Harsányi L, Kittel Á, Gézsi A, *et al*: Shared extracellular vesicle miRNA profiles of matched ductal pancreatic adenocarcinoma organoids and blood plasma samples show the power of organoid technology. *Cell Mol Life Sci* 78: 3005-3020, 2021.
95. Zhang Y, Lu A, Zhuang Z, Zhang S, Liu S, Chen H, Yang X and Wang Z: Can organoid model reveal a key role of extracellular vesicles in tumors? A comprehensive review of the literature. *Int J Nanomedicine* 18: 5511-5527, 2023.
96. Zhou G, Li R, Sheng S, Huang J, Zhou F, Wei Y, Liu H and Su J: Organoids and organoid extracellular vesicles-based disease treatment strategies. *J Nanobiotechnology* 22: 679, 2024.
97. Crescitelli R, Lässer C and Lötvall J: Isolation and characterization of extracellular vesicle subpopulations from tissues. *Nat Protoc* 16: 1548-1580, 2021.
98. Zhang C, Yang X, Jiang T, Yan C, Xu X and Chen Z: Tissue-derived extracellular vesicles: Isolation, purification, and multiple roles in normal and tumor tissues. *Life Sci* 321: 121624, 2023.
99. Serrati S, Di Fonte R, Porcelli L, De Summa S, De Risi I, Fucci L, Ruggieri E, Marvulli TM, Strippoli S, Fasano R, *et al*: Circulating extracellular vesicles are monitoring biomarkers of anti-PD1 response and enhancer of tumor progression and immunosuppression in metastatic melanoma. *J Exp Clin Cancer Res* 42: 251, 2023.
100. Guo J, Jiang G, Chen J, Zhang M, Xiang K, Wang C, Jiang T, Kang Y, Sun Y, Xu X, *et al*: Tumor tissue derived extracellular vesicles promote diabetic wound healing. *J Diabetes Complications* 37: 108435, 2023.
101. Sprangers J, Zaalberg IC and Maurice MM: Organoid-based modeling of intestinal development, regeneration, and repair. *Cell Death Differ* 28: 95-107, 2021.
102. Strobel HA, Moss SM and Hoying JB: Methods for vascularization and perfusion of tissue organoids. *Mamm Genome* 33: 437-450, 2022.
103. Lai Benjamin FL, Lu Rick X, Hu Y, Davenport HL, Dou W, Wang EY, Radulovich N, Tsao MS, Sun Y and Radisic M: Recapitulating pancreatic tumor microenvironment through synergistic use of patient organoids and organ-on-a-chip vasculature. *Adv Funct Mater* 30: 2000545, 2020.
104. Zheng F, Xiao Y, Liu H, Fan Y and Dao M: Patient-specific organoid and organ-on-a-chip: 3D cell-culture meets 3D printing and numerical simulation. *Adv Biol (Weinh)* 5: e2000024, 2021.
105. Hu Y, Zhu T, Cui H and Cui H: Integrating 3D bioprinting and organoids to better recapitulate the complexity of cellular microenvironments for tissue engineering. *Adv Healthc Mater* 14: e2403762, 2025.



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