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From biomarkers to drug delivery: advances in extracellular vesicles in cancer diagnosis and targeted therapy

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Abstract

Extracellular vesicles (EVs), lipid-bilayer nanoparticles secreted by diverse cell types, have emerged as pivotal mediators in cancer progression through intercellular communication. These vesicles encapsulate oncogenic biomolecules—including proteins, nucleic acids, and metabolites—enabling the reprogramming of recipient cells to foster tumor initiation, angiogenesis, immune evasion, and metastatic niche formation. Tumor-derived EVs (TDEVs) reflect parental cell molecular profiles, offering non-invasive biomarkers for cancer diagnosis. Their stability in bodily fluids and enrichment of tumor-specific markers, such as mutant DNA, oncogenic miRNAs, and immunosuppressive ligands, surpass conventional circulating biomarkers in sensitivity and specificity, positioning EVs as promising tools for liquid biopsy. In addition to their diagnostic potential, EVs have dual roles as therapeutic targets and drug delivery vehicles. TDEVs promote chemoresistance by transferring drug efflux pumps, pro-survival RNAs, and immunosuppressive factors, but their inherent biocompatibility and targeting capabilities make them ideal nanocarriers. In preclinical models, engineered EVs loaded with chemotherapeutics, siRNA, or CRISPR-Cas9 components exhibit enhanced tumor accumulation and reduced off-target toxicity. Surface modifications, such as peptide conjugation or antibody integration, further improve their homing specificity. This review synthesizes advances in EV biology, diagnostic applications, and therapeutic innovations, highlighting their translational potential in oncology. By combining mechanistic insights with translational opportunities, we highlight the evolving promise of EVs as versatile platforms for cancer management.

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KEYWORDS

Extracellular vesicle; Cancer; Biomarkers; Tumor progression; Targeted therapy

Introduction

Extracellular vesicles (EVs) are nanoparticles encapsulated by a lipid bilayer that are secreted by almost all cell types under physiological and pathological conditions. These vesicles include exosomes, microvesicles, and apoptotic bodies, which vary in size, biogenesis, and molecular content. As vehicles for intercellular communication, EVs carry a variety of bioactive substances, such as proteins, lipids, DNA, mRNA, and noncoding RNA, thereby enabling horizontal transfer of biological information(1). Through this mode of transport, EVs regulate a variety of cellular processes, including immune regulation, tissue repair, and metabolic homeostasis. Their inherent stability, biocompatibility, and ability to cross biological barriers make them extremely attractive for diagnostic and therapeutic applications(2).

Tumor progression is a multifaceted process regulated by complex intercellular communication within the tumor microenvironment (TME)(3). Among the many mediators involved in such communication, EVs have emerged as key messengers that promote local and systemic signaling. In oncology, tumor-derived extracellular vesicles (TDEVs) are involved in almost every stage of cancer progression. By transferring oncogenic molecules to neighboring or distant cells, TDEVs promote tumor initiation, enhance angiogenesis, promote immune escape, and support the formation of a metastatic microenvironment(4). EVs derived from stromal or immune cells can modulate tumor growth and therapeutic response, thereby constructing a dynamic bidirectional communication network(5). In addition to their biological functions, EVs have shown potential as non-invasive biomarkers due to their easy expression in various body fluids and their ability to reflect the molecular characteristics of parental cells(6). In addition, their natural origin and ability to encapsulate functional molecules have also prompted the development of EV-based drug delivery platforms in preclinical cancer models(7).

Over the past decade, the study of EVs in oncology has developed rapidly. A large number of studies have elucidated the mechanisms by which EVs mediate intercellular communication, influence cancer characteristics, and modulate therapeutic sensitivity. Despite these advances, several challenges remain, including the standardization of EVs isolation methods, the elucidation of in vivo biodistribution, and the optimization of engineering strategies for therapeutic use(8). This review aims to provide a comprehensive overview of the role of extracellular vesicles in cancer biology and treatment. We summarize recent research findings on the biogenesis, development, and functional impact of EVs in various cancer types. In addition, we discuss recent advances in the clinical application of EVs as diagnostic tools and therapeutic vectors. By highlighting key challenges and future directions, we aim to gain insight into the translational potential of EVs in oncology and to facilitate further research in this rapidly evolving field.

1.Biological characteristics and formation mechanism

EV formation involves complex and tightly regulated cellular pathways. The main ways for cells to take up extracellular substances include plasma membrane invagination and endocytosis. During intracellular transport, early sorting endosomes (ESEs) gradually transform into late sorting endosomes (LSEs) by fusing with vesicles. The secondary

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invagination of the LSE membrane structure generates intraluminal vesicles (ILVs), at which point the organelle evolves into multivesicular bodies (MVBs). As the mature form of LSE, multivesicular bodies (MVBs) have three fates: degradation of contents through fusion with lysosomes or autophagosomes; or release of their internal vesicles after fusion with the plasma membrane. These released ILVs are called small extracellular vesicles. In addition, there are other formation mechanisms for extracellular vesicles: the formation mechanism of microvesicles involves outward budding and fission of the plasma membrane, while apoptotic bodies are large membrane vesicle structures produced during programmed cell death(9) (Figure 1).

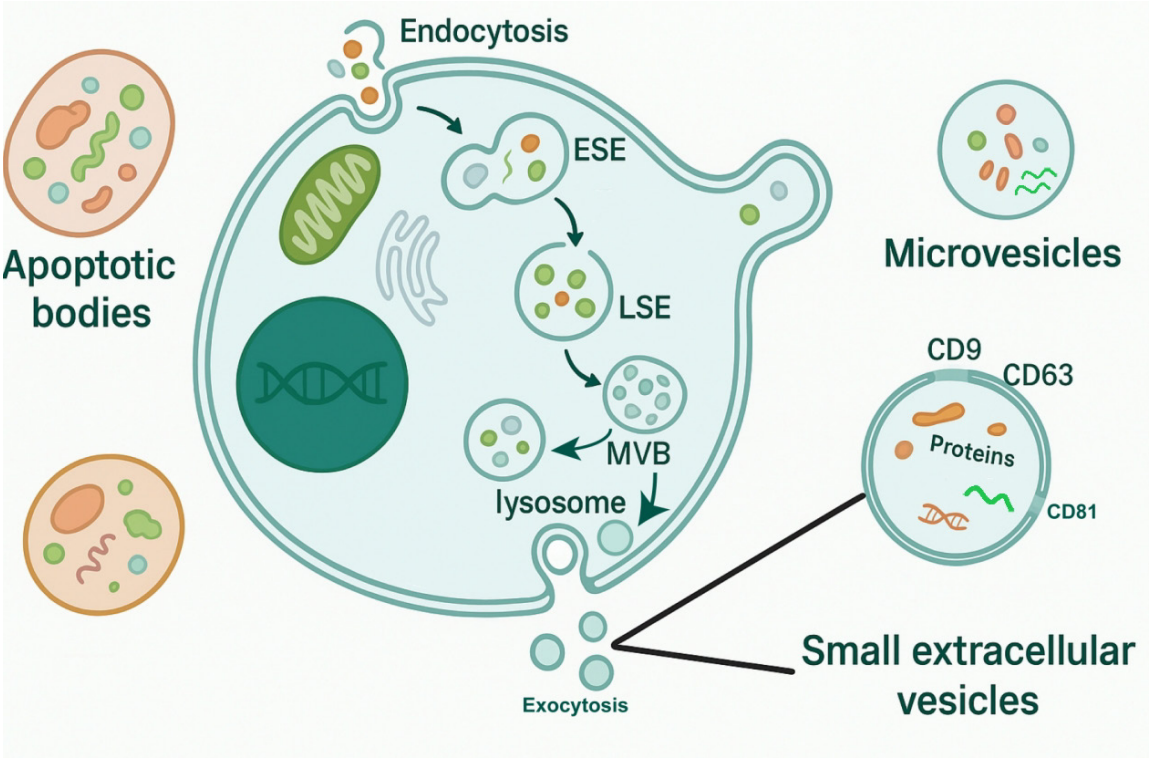

FIGURE 1
Sorting and biogenesis of EVs.

The formation of LSEs originates from the vesicle conversion process of fusion with ESEs. Its ILVs are generated by the secondary membrane invagination process of LSEs. LSEs further evolve into MVBs. MVBs degrade their contents by fusion with lysosomes or autophagosomes, or release ILVs after fusion with the plasma membrane. ILVs released by this pathway are defined as small extracellular vesicles. The formation mechanism of microvesicles involves the outward protrusion and rupture of the plasma membrane, while apoptotic bodies are large membrane vesicles generated during programmed cell death. EVs: Extracellular vesicles; LSEs: Late sorting endosomes; ESEs: Early sorting endosomes; ILVs: Intraluminal vesicles; MVBs: Multivesicular bodies.

EV release is tightly regulated by cellular energy status, environmental stimuli, and molecular switches. Exosome secretion involves the transport of multivesicular bodies along microtubules to the plasma membrane, a process mediated by Rab GTPases (Rab27a/b, Rab11). Rab27a knockdown significantly reduces exosome release by inhibiting MVB docking(10). Microvesicle shedding requires

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activation of ADP-ribosylation factor 6 (ARF6), which coordinates extracellular signal-regulated kinase (ERK) signaling and myosin light chain kinase (MLCK) activity(11). TME stressors, such as hypoxia and nutrient deprivation, upregulate EV secretion through a hypoxia-inducible factor 1 α (HIF-1α)-dependent pathway, thereby enhancing the delivery of pro-tumorigenic substances. Notably, sphingomyelinase 2 (nSMase2), a key enzyme in ceramide synthesis, is its pharmacological target; GW4869-mediated inhibition of nSMase2 effectively inhibits exosome release in multiple cancer models(12).

EVs selectively encapsulate proteins, nucleic acids (mRNA, miRNA, lncRNA), lipids, and metabolites through mechanisms such as ubiquitination, RNA-binding proteins, and lipid raft microdomains(13). Tetraspanin-rich microdomains promote the aggregation of integrins and metalloproteinases into exosomes, while nuclear heterogeneous ribonucleoproteins (hnRNPA2B1, YBX1) recognize miRNA-specific motifs (e.g. GGAG in miR-223) for RNA loading. Tumor-derived EVs often carry oncogenic miRNAs (miR-21, miR-155), mutant oncoproteins (EGFRvIII, KRASG12D), and immunosuppressive ligands (PD-L1) that reprogram recipient cells to promote metastasis and immune escape(14). Lipidomic analysis showed that EVs are enriched in cholesterol, sphingomyelin, and phosphatidylserine compared with the parental membrane. This lipid composition not only stabilizes the vesicle structure but also promotes cellular uptake through membrane fusion or endocytosis(15). Metabolic enzymes (pyruvate kinase M2, hexokinase) encapsulated in EVs may also reshape the energy metabolism of stromal cells in the pre-metastatic microenvironment(16).

2.Regulatory roles of EVs in tumor pathogenesis

Tumor-derived EVs carry a diverse array of bioactive molecules, such as proteins, nucleic acids, lipids, and metabolites, reflecting the molecular characteristics of their originating cells. Increasing evidence indicates that EVs are not merely byproducts of cellular activity, but instead play dynamic regulatory roles in the initiation, progression, and dissemination of cancer. In the early stages of tumorigenesis, EVs contribute to malignant transformation by transferring oncogenic factors to surrounding or distant cells. Genetic materials, such as mutant KRAS mRNA, amplified MYC transcripts, or deregulated microRNAs, can be horizontally delivered via EVs, thereby reprogramming recipient cells toward a tumor-promoting phenotype(17). Moreover, EVs have been shown to modulate signaling pathways such as PI3K/AKT, MAPK, and Wnt/β-catenin, creating a pro-survival and proliferative environment conducive to neoplastic growth(18). Beyond tumor initiation, EVs play critical roles in promoting angiogenesis, a hallmark of cancer that supports the increasing metabolic demands of growing tumors. Tumor-secreted EVs are enriched in pro-angiogenic factors, including VEGF, IL-8, and angiopoietin-like proteins, which can activate endothelial cells and stimulate neovascularization(19). Furthermore, EVs from hypoxic tumor regions contain distinct molecular cargo that enhances vascular permeability and endothelial cell migration, facilitating the establishment of an abnormal yet functionally supportive vasculature(20).

TME represents a complex ecosystem where malignant cells interact with immune cells, fibroblasts, endothelial cells, and extracellular matrix components(21). EVs serve as key communicators in this crosstalk, mediating immunosuppression, stromal remodeling, and metabolic reprogramming. For instance, tumor-derived EVs can induce the polarization of macrophages toward an M2 phenotype, suppress cytotoxic T cell activity, and impair natural killer cell function, thereby fostering immune evasion(22). Simultaneously, EVs influence cancer-associated fibroblasts and mesenchymal stem cells, promoting the secretion of matrix-degrading enzymes and growth factors that facilitate invasion and metastasis(23). Given their integral roles in multiple stages of tumor pathogenesis, EVs have garnered increasing attention as potential diagnostic biomarkers and therapeutic targets. The following sections will provide a detailed exploration of the mechanisms through which EVs regulate tumor development, angiogenesis, and TME remodeling, thereby underscoring their multifaceted contributions to cancer biology and translational potential in oncology.

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2.1 Modulation of tumor initiation and progression

EVs have become key mediators of intercellular communication in the TME, coordinating complex regulatory networks and driving the development of tumors such as occurrence and metastasis. These nanoparticles encapsulate a variety of bioactive molecules, including proteins, nucleic acids, and metabolites, enabling them to reprogram recipient cells by horizontally transferring carcinogens. In the early carcinogenesis process, tumor-derived EVs transfer oncogenic proteins, miRNAs, and metabolites, thereby inducing malignant transformation of adjacent normal cells. For example, small EVs from breast cancer regulate tumor progression-related signaling pathways by phosphorylating the proteome, while exosomal miR-484 promotes pancreatic cancer cell proliferation and metastasis by inhibiting target genes VEGFR2 and BEX1(24). In small cell lung cancer with MYC gene amplification, EVs deliver MYC-dependent signaling molecules (such as miRNAs and proteins) to activate the proliferation of recipient cells(25). Oncogene-related EVs can also promote tumor initiation by transferring integrins, Wnt signaling molecules, etc(26).

Tumor progression is also regulated by crosstalk between malignant cells and matrix components promoted by EVs. EVs remodel the extracellular matrix (ECM) by delivering matrix metalloproteinases (MMPs) and fibronectin, thereby enhancing invasive potential(27). Phosphorylated proteins in breast cancer EVs enhance cell migration ability by activating the Rho-GTPase signaling pathway(28). In addition, EVs establish an immunosuppressive microenvironment through PD-L1 presentation and TGF-β secretion, effectively inhibiting cytotoxic T cell responses. For example, melanoma EVs can promote immune escape by inhibiting dendritic cell maturation and cytotoxic T cell function(29). Metastatic progression depends largely on EVs-mediated priming of distant organs. Primary tumors release EVs to induce secondary tumor sites by inducing vascular leakage, fibroblast activation, and immune cell recruitment(30). Tumor EVs target specific organs (lungs and liver) through integrins (such as α6β4, αvβ5), induce local stromal cells to secrete chemokines (such as S100A8/A9), and provide a suitable microenvironment for metastatic cells(31, 32). Interestingly, EV cargo shows temporal heterogeneity during disease progression. Advanced non-small cell lung cancer EVs exhibited elevated levels of ANXA2, which promotes angiogenesis and EMT in metastatic lesions(31).

3.2 Modulation of angiogenesis and TME

The TME comprises a complex network of cancer-associated fibroblasts (CAFs), immune cells, and extracellular matrix (ECM) components, all of which are susceptible to EV-mediated regulation. Tumor EVs induce CAFs to develop a pro-tumorigenic phenotype characterized by enhanced secretion of TGF-β, IL-6, and ECM remodeling enzymes. In turn, CAF-derived EVs enrich tumor cells with metabolites (e.g. glutamine) and survival signals (e.g. lncRNAs that activate STAT3), thereby establishing a feed-forward loop of matrix activation(33). CAF-EVs in gastric cancer induce macrophage M2 polarization through the miR-139-5p/JAK1/STAT3 axis and promote tumor progression(34). EV-associated PD-L1 can also induce T cell exhaustion, thereby enhancing immune evasion. In colorectal cancer liver metastasis, tumor-associated macrophage-derived extracellular vesicles (TAM-EVs) downregulate T cell receptor signaling through miR-21-5p and miR-29a-3p, promoting immunosuppression(35). EV-packaged TGF-β and IL-10 polarize macrophages toward the immunosuppressive M2 phenotype. In addition, EV-mediated ECM stiffening promotes the formation of a metastatic microenvironment through collagen cross-linking enzymes (LOX, LOXL2), creating conditions for tumor cell invasion(36).

EVs derived from tumor cells promote angiogenesis by transferring pro-angiogenic factors (such as VEGF, FGF, and angiopoietin) to endothelial cells, thereby stimulating endothelial cell proliferation, migration, and tubulogenesis. Tumor-derived EVs carry molecules such as miR-21 and miR-29a, which induce macrophages to polarize toward the pro-tumor M2 phenotype, promoting angiogenesis and matrix remodeling(37). Hepatocellular carcinoma EVs in the hypoxic microenvironment activate the STAT3 pathway through the ANXA1 protein, enhancing the M2 polarization of TAMs(38). TGFβ in EVs derived from head and neck squamous cell carcinoma enhances angiogenic activity through the SMAD signaling pathway(39). In addition to directly delivering angiogenic proteins, EVs can also transfer miRNAs targeting anti-angiogenic pathways. The miRNAs carried by EVs (such as miR-21, miR-210, etc.) are delivered

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to endothelial cells (ECs), directly promoting endothelial cell proliferation, migration and lumen formation by inhibiting anti-angiogenic genes or activating pro-angiogenic signaling pathways (such as PI3K/Akt). In addition, hypoxic conditions in the TME enhance the release of EVs and change their molecular structure, thereby enhancing the pro-angiogenic phenotype of recipient endothelial cells and promoting vascular remodeling(40).

3.Application of EVs in tumor diagnosis

The mechanism of EVs as tumor-specific biomarkers mainly involves their multifunctional role in tumorigenesis, metastasis and immune regulation, as well as the tumor-specific molecular features they carry. EVs derived from tumor cells or TME can encapsulate molecular features reflecting oncogenic changes, making them superior to traditional circulating biomarkers. EVs are involved in the delivery mechanism of tumor-specific molecules. EVs secreted by tumor cells are rich in tumor-related proteins (such as PD-L1, EGFR), nucleic acids (miRNA, lncRNA, mutant DNA) and lipids, which can reflect the genetic mutations, metabolic status and drug resistance of the tumor(41). For example, EVs derived from skin tumors were detected in patients with renal cell carcinoma to carry tumor-promoting molecules and enhance cancer cell proliferation(42). In terms of transcellular transmission of oncogenic signals, EVs deliver their contents to recipient cells through endocytosis or membrane fusion, activating pro-metastatic pathways (such as EMT, angiogenesis) or inhibiting immune responses. For example, EVs derived from breast cancer regulate autophagy and tumor microenvironment remodeling through miRNA(43). DNA fragments in apoptotic bodies carry tumor-specific mutations (such as EGFR T790M, KRAS G12V) and can be used for gene mutation analysis in liquid biopsy(44). Detection of EGFR mutations in apoptotic bodies in patients with non-small cell lung cancer has been shown to be highly consistent with tissue biopsy results(45).

In addition, the application of EVs in liquid biopsy has great advantages. EVs exist in body fluids such as blood and urine and can be obtained non-invasively. The tumor-specific markers it carries (such as EGFR mutations and KRAS mutations) are more stable and highly enriched than circulating tumor DNA (ctDNA). For example, tumor-derived EVs contain tumor-specific proteins (such as CD99, ENO-1) and mutant genes, which can be used for early diagnosis of cancer and efficacy monitoring. Studies have found that co-expression of exosome surface markers (such as CD63, CD9) and tumor-related proteins (such as EpCAM, HER2) can improve detection specificity(46). EVs regulate the physiological functions of recipient cells by transmitting signal molecules (such as miRNA, mRNA, and metabolic enzymes), and participate in TME remodeling, pre-metastatic niche formation, and drug resistance transmission. For example, EVs from patients with acute myeloid leukemia enhance the mitochondrial function of leukemia cells through the glutathione/GPX4 axis, promoting disease progression(47). EVs are widely present in body fluids such as blood, urine, and saliva. Their abundance is higher than that of circulating tumor cells (CTCs), and they can overcome the limitations of ctDNA fragmentation and low concentration, providing a more comprehensive tumor molecular spectrum.

4. Potential of EVs in cancer therapy

The ubiquitous presence of EVs in body fluids and their inherent biocompatibility make them promising candidates for advancing cancer diagnostics and treatment. Notably, the dual role of EVs in tumor progression—promoting oncogenic signaling while also possessing therapeutic potential—has fueled intense interest in their clinical applications(48). This

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dichotomy highlights the urgency of elucidating the mechanisms of EV biogenesis and improving their application in oncology, especially as conventional chemotherapy continues to face challenges such as systemic toxicity and multidrug resistance.

A key obstacle to effective cancer therapy is chemoresistance, a phenomenon that is increasingly associated with EV-mediated cell-to-cell crosstalk. Tumor-derived EVs have been shown to participate in the transfer of drug efflux pumps, pro-survival miRNAs, and anti-apoptotic factors to recipient cells, thereby conferring chemoresistance in heterogeneous tumor populations. For example, exosome transfer of P-glycoprotein and multidrug resistance-associated protein has been shown to reduce drug accumulation within sensitive cells(49). Furthermore, EVs secreted by stromal cells in the tumor microenvironment can activate pro-survival pathways in malignant cells, such as PI3K/Akt and NF-κB, thereby exacerbating resistance to therapy(50). These findings not only highlight EVs as a causative factor for treatment failure, but also suggest their potential as drug targets that can reverse the resistance phenotype.

In addition to their pathophysiological roles, EVs have intrinsic properties that make them ideal vehicles for therapeutic intervention. Their natural ability to cross biological barriers, evade immune clearance, and deliver drugs to specific cell types transcends the limitations of synthetic nanoparticles. This has prompted the development of innovative strategies to transform EVs into precision-targeted drug delivery systems. Recent advances include loading EVs with chemotherapeutic drugs, small interfering RNA (siRNA), or CRISPR-Cas9 components by electroporation, sonication, or genetic modification of parental cells(51). For example, doxorubicin-loaded exosomes derived from mesenchymal stem cells have been shown to have enhanced tumor accumulation and reduced cardiotoxicity compared to free drug preparations(52). Meanwhile, surface engineering techniques such as peptide insertion or antibody conjugation can tailor the tropism of EVs, thereby improving delivery efficiency to malignant tissues while sparing healthy areas.

Therapeutic targeting of EVs themselves represents another frontier in oncology. Strategies such as inhibiting EV biogenesis by knocking down Rab27a or disrupting EV uptake by blocking surface integrins have shown efficacy in preclinical models(53). Drugs targeting nSMase2, a key enzyme in exosome formation, are being evaluated for their ability to attenuate EV-mediated metastasis. However, vesicles that propagate drug resistance may be repurposed as biomarkers to monitor treatment response or as immunomodulators when loaded with tumor antigens to stimulate anticancer immunity(54). Despite these advances, the clinical translation of EV-based therapies still requires addressing some key challenges. Standardization of isolation protocols, scalability of production, and rigorous evaluation of off-target effects remain critical to ensure the reproducibility and safety of treatments.

4.1 Mechanisms mediating chemoresistance

Chemoresistance remains a formidable challenge in oncology, often leading to tumor recurrence and treatment failure. EVs are key mediators of chemoresistance through multiple mechanisms, facilitating the transfer of bioactive molecules that contribute to resistance. The primary mechanism by which EVs promote chemoresistance involves direct efflux of chemotherapeutic drugs. Tumor-derived EVs often encapsulate ATP-binding cassette (ABC) transporters(55). For example, EVs carry transporters such as P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP), which can enhance chemotherapeutic drug efflux when transferred to sensitive tumor cells. These proteins are overexpressed in resistant cells. Upon EV uptake by recipient cells, these transporters integrate into the plasma membrane, actively expel cytotoxic drugs and reduce intracellular drug accumulation(56). Transfer of exosomal P-gP has been shown to reduce the efficacy of doxorubicin in breast cancer models, recapitulating the resistant phenotype in previously sensitive cells. This horizontal transfer of resistance-associated proteins highlights the role of EVs as carriers of functional molecular mechanisms.

In addition to drug efflux, EVs mediate chemoresistance by delivering nucleic acids that modulate survival pathways. Tumor-derived exosomes enriched in miRNAs, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) can reprogram recipient cells to evade apoptosis. Exosomal miR-21-5p inhibits phosphatase and tensin homolog (PTEN) expression, thereby activating the PI3K/Akt pathway and

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enhancing cell survival in ovarian cancer patients under cisplatin treatment(57). Similarly, lncRNA H19 packaged in EVs activates the Wnt/β-catenin signaling cascade in hepatocellular carcinoma, thereby promoting resistance to sorafenib(58). These findings highlight that nucleic acids carried by EVs can modulate pro-survival signaling networks. The TME can further promote EV-mediated chemoresistance through stroma-tumor crosstalk. CAFs secrete EVs containing metabolites and growth factors that can maintain tumor cell viability under chemotherapeutic stress. In pancreatic ductal adenocarcinoma, CAF-derived exosomes transport deoxycytidine kinase inhibitors, which impair gemcitabine metabolism and confer resistance(59). In addition, exosomes from hypoxic tumor areas carry hypoxia-inducible factor-1α (HIF-1α), which induces angiogenesis and upregulates drug efflux pumps in endothelial cells. Emerging evidence suggests that EVs are involved in inducing cancer stem cell (CSC)-like properties that are intrinsically resistant to chemotherapy. In lung cancer and melanoma, EVs have been found to deliver stemness-associated transcription factors such as Oct4 and Nanog or lncRNAs such as HOTAIR, which promote recipient cells to acquire a stem cell-like phenotype and enhance chemotherapy resistance(60). Finally, EVs facilitate immune evasion and indirectly promote chemoresistance by suppressing antitumor immunity. Tumor-derived exosomes carrying programmed death ligand 1 (PD-L1) can inhibit T cell activation, while EVs enriched in TGF-β can induce the expansion of regulatory T cells.

4.2 As therapeutic targets or drug delivery vehicles

Almost all cells can secrete EVs, which carry bioactive molecules such as proteins, nucleic acids, and lipids and participate in intercellular communication, making them delivery vehicles for chemotherapeutic drugs, RNA drugs, and gene editing tools. Abnormal secretion of EVs by tumor cells promotes tumor metastasis, immune escape, and therapeutic resistance by transferring oncogenic proteins, nucleic acids, and metabolites to recipient cells. Targeting the biosynthesis or uptake mechanism of EVs is a feasible strategy to slow tumor progression. Inhibition of Rab GTPases (key regulators of multivesicular body formation) has been shown to inhibit EV release in glioblastoma models, thereby reducing tumor invasiveness(61). Similarly, drug blockade of neutral sphingomyelinase 2 (nSMase2, an enzyme critical for exosome cargo sorting) can reduce breast cancer metastasis. Neutralizing antibodies targeting EV surface tetraspanins (e.g. CD9, CD63) or integrins (e.g. αvβ3) can effectively disrupt EV-mediated crosstalk between tumor cells and matrix components, thereby inhibiting angiogenesis and the formation of an immunosuppressive microenvironment.

Compared with synthetic nanoparticles, EVs have natural advantages, including excellent biocompatibility, low immunogenicity, and the ability to penetrate biological barriers(62). Tumor cell-derived EVs carry parental cell-specific proteins (such as integrins and transmembrane proteins) on their surface, which can preferentially target homologous tumor cells and deliver drugs through endocytosis or membrane fusion. By designing EVs to encapsulate therapeutic drugs (such as chemotherapeutic drugs, siRNA or immune checkpoint inhibitors), tumor-targeted delivery can be enhanced while minimizing systemic toxicity. For example, small EVs (sEVs) derived from breast cancer cells carry specific phosphorylated proteins and can be used as liquid biopsy markers. In studies of glioblastoma cells, stem cell-derived EVs can cross the blood-brain barrier and deliver temozolomide (TMZ) to the core area of the tumor(63). Oral cancer cell EVs overexpress EGFR antibodies on their surface, which can specifically target EGFR-highly expressing tumors and deliver erlotinib(64). Emerging studies have also reported the combined use of EVs with immune checkpoint inhibitors or radiotherapy, suggesting synergistic therapeutic benefits. In particular, dendritic cell-derived EVs carrying tumor antigens have been used as cancer vaccines to stimulate anti-tumor immunity. Some of these EV-based vaccines have entered early clinical trials, reinforcing the translational application value of EV research.

4.3 Engineered drug delivery system based on EVs

EVs have emerged as promising nanocarriers for drug delivery due to their inherent biocompatibility, low immunogenicity, and natural ability to penetrate biological barriers. EVs have a lipid bilayer membrane derived from the parental cells that protects their drug cargo and facilitates targeted delivery. Recent advances in EV engineering have

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further enhanced their potential as a therapeutic platform, enabling precise drug delivery, improved stability, and enhanced tumor targeting. EVs carry membrane proteins from parental cells on their surface, allowing them to target tumor cells or tumor-associated stromal cells through ligand-receptor interactions. TDEVs have homing abilities similar to parental cells, can penetrate the vascular barrier, and accumulate in tumor tissues(65). Stem cell-derived EVs, on the other hand, take advantage of their immune escape properties and tumor homing ability to preferentially accumulate at tumor sites. Drug encapsulation in EV engineering can be achieved using both passive and active approaches. Passive approaches, such as co-incubation with hydrophobic chemotherapeutic drugs such as doxorubicin or paclitaxel, utilize the lipid bilayer structure of EVs to promote drug diffusion(66). Multifunctional sEVs achieved chemo-immunotherapy in a lung cancer model by simultaneously delivering paclitaxel and PD-L1 siRNA, resulting in a 68% reduction in tumor volume. Active loading techniques, including electroporation, sonication, and freeze-thaw cycles, can improve encapsulation efficiency but may compromise membrane integrity if not optimized properly. Chemotherapeutic drugs (e.g. doxorubicin), nucleic acids (siRNA/miRNA), or CRISPR/Cas systems are loaded into the EV lumen by electroporation, sonication, or co-incubation(67). Recent innovations, such as pH gradient-driven loading and CRISPR-Cas9-mediated gene editing of parental cells, allow nucleic acids (siRNA, miRNA) or proteins (enzymes, antibodies) to be precisely incorporated into EVs without causing structural damage.

Conclusion

EVs have emerged as key mediators of intercellular communication within the TME, regulating tumorigenesis, progression, and treatment prognosis through the horizontal transfer of oncogenic biomolecules. Their ability to encapsulate tumor-specific proteins, nucleic acids, and lipids reflects the molecular architecture of the parental cells, which offers unparalleled advantages over traditional circulating biomarkers. In addition, they reshape the TME by inducing angiogenesis, immune evasion, and stromal reprogramming, highlighting their central role in tumor pathogenesis. The molecular cargo of EVs, including oncoproteins, metastatic miRNAs, and immunosuppressive cytokines, drives the establishment of a pre-metastatic microenvironment and chemoresistance. Tumor-derived EVs use integrin-mediated organ tropism to prepare for distant metastasis, while stromal cell-derived EVs promote resistance through metabolic reprogramming and survival signaling.

The inherent biocompatibility, tumor homing, and ability to cross biological barriers of EVs surpass the limitations of synthetic nanoparticles. Several engineering strategies, such as surface modification with targeting ligands or loading with chemotherapeutic drugs, siRNA, or CRISPR-Cas9 systems, have been shown to enhance tumor specificity and reduce off-target toxicity. The success of preclinical studies, including mesenchymal stem cell-derived EVs for doxorubicin delivery and dendritic cell-derived EVs as cancer vaccines, highlights their translational potential. Combining EV-based therapies with conventional therapies may result in synergistic antitumor effects. As the field advances, rigorous clinical validation of EV biomarkers and therapeutic platforms will be critical to translating these discoveries into personalized cancer management strategies. In summary, EVs embody the complexity of tumor biology, and their dual properties as biomarkers and therapeutic agents highlight their transformative potential in oncology.

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