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REVIEW

Engineered plant extracellular vesicles: Emerging nanoplatforms for combinational cancer immunotherapy

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Abstract Plant-derived extracellular vesicles (PDEVs), describe a group of nanoparticles released by plants. These particles are characterized by a lipid bilayer structure containing various proteins, lipids, nucleic acids, and unique metabolites. Although the study on PDEVs is relatively new, having only been around for ten years, they have shown promising development prospects in both basic research and clinical transformation areas. Evidence suggests that PDEVs have excellent application prospects in regulating inflammation and treating tumors. Their distinctive, vesicle-mimicking architecture and stellar biocompatibility render them prime candidates for ferrying various anti-cancer agents, including RNA, proteins, and conventional chemotherapy drugs. Increasingly, studies have shown that PDEVs can be engineered as an innovative platform for combination cancer immunotherapy. Consequently, this paper provides an extensive summary of current developments in engineering methods and strategies for PDEVs in cancer treatment and combined cancer immune therapeutics. The essential characteristics of PDEVs, including the biogenesis process and components, as well as their anti-tumor activity and mechanism, are summarized. Finally, the *in vivo* safety of PDEVs as delivery vectors and the challenges of scale-up production and clinical transformation are discussed.

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1. Introduction

Animal and plant cells maintain communication between cells by secreting various signaling substances, a fundamental biological function essential for growth and development¹. For animal and plant cells, the secretion of extracellular vesicles (EVs) can affect the growth and development of adjacent and distant cells. EVs are membranous vesicle substances released by animal and plant cells, including micro-vesicles (MVs), exosomes, and apoptotic bodies²⁻⁴. These EVs participate in cellular life processes and are widely distributed in various bodily fluids and cells^{5,6}. Over the past decade, researchers have conducted extensive basic and preclinical studies on mammalian-derived extracellular vesicles (MDVs), which play a crucial role in cancer treatment. Tumor-derived EVs can drive tumor growth, angiogenesis, invasive growth, immune escape, treatment tolerance, and postoperative recurrence⁷. Researchers have developed some autologous EVs based on tumor cell origin and achieved good clinical results by focusing on the unique properties of tumor-derived EVs⁸. A mature theoretical system has been established for the biogenesis, composition, and tumor application of mammalian electric vesicles^{9,10}.

Plant-derived extracellular vesicles (PDEVs) are nanoscale membrane vesicles secreted by plant cells, widely present in various plant tissues (*e.g.*, leaves, fruits, roots) and secretions¹¹. In terms of structure, they have a lipid bilayer membrane and contain a variety of bioactive substances, including proteins, lipids, polyphenols, flavonoids, vitamins, and genetic materials¹². More significantly, new research highlights the substantial potential for cross-species applications of PDEVs, which mediate intercellular communication, material transport, and environmental stress responses in plants¹³. PDEVs are classified as natural disease therapeutics and delivery vehicles offering several advantages, including ease of use, safety, environmental friendliness, affordability, and low toxicity due to their high yield, low risk of immunogenicity *in vivo*, and excellent biocompatibility, modifiability, and ability to encapsulate functional molecules¹²⁻¹⁴. Although the development of PDEVs is still nascent, numerous foundational studies have revealed PDEVs' significant potential in preventing diseases and delivering drugs over the past ten years¹⁵⁻¹⁷. PDEVs have demonstrated promising prospects in the clinical transformation of nanotherapeutics. Numerous basic types of research have shown that PDEVs can exert therapeutic effects in different tumors, such as lung carcinoma, glioblastoma, colon cancer, breast cancer, and hepatocellular carcinoma¹⁶⁻²². The bioactive compounds found in PDEVs can curb tumor progression through multiple mechanisms¹⁸⁻²². These small molecules can modulate immune cell activity, drive macrophages toward an M1 phenotype, suppress cellular proliferation, induce programmed cell death, and even counteract drug resistance in cancer cells. PDEVs have also shown desirable prospects in drug delivery platform construction. The nanoscale size makes them excellent for tissue penetration and accumulation^{23,24}. The low toxicity and lipid bilayer structure make them biocompatible physiologically²⁵. These advantages make PDEVs a superior nanocarrier to synthetic carriers and MDVs²⁴. They can be embellished and

engineered to carriers to achieve targeted delivery, improve curative effects, and reduce systemic toxicity²⁴.

Increasingly, engineering strategies are being used to modify PDEVs^{26,27}. These modification methods mainly include surface engineering methods and membrane fusion technology²⁸. Surface engineering refers to changing the surface of PDEVs, primarily involving chemical and physical alterations. The chemical insertion method mostly modifies the functionality of molecules to the surface of PDEVs through chemical reactions, thereby endowing PDEVs with tumor targeting ability and tissue penetration ability^{29,30}. The physical method mainly refers to physical and static point adsorption to modify the decoration on the surface of PDEVs, which is more straightforward than the chemical insertion physical modification method³⁰⁻³². Membrane fusion modification technology refers to the membrane fusion of vesicles containing PDEVs and cell membranes or membrane-like structures under specific conditions, thereby enhancing the function of PDEVs^{33,34}. For example, PDEVs can be fused with tumor cells or immune cell membranes to achieve tumor targeting or tumor microenvironment targeting of PDEVs³⁵.

Tumor immunotherapy refers to the induction of tumor regression through immune response, a promising anti-tumor method in clinical practice^{36,37}. However, the immunosuppressive microenvironment of the tumor, non-therapeutic side effects, and tumor immune escape significantly limit its clinical application³⁸. Recent studies indicate that integrating immunotherapy with alternative treatments enhances anti-cancer efficacy, suppressing both tumor expansion and relapse³⁶. However, selecting the right drug carrier to co-deliver drugs of different therapies to the tumor site remains a significant challenge^{36,37}. As a natural drug delivery vector, PDEVs can be engineered into functional drug delivery systems to achieve multi-drug co-delivery and combined immunotherapy^{5,26}. In addition, some PDEVs with immunomodulatory properties can synergistically enhance the efficacy of combined immunotherapy^{39,40}. The *in vivo* biosafety of PDEVs is a key barrier to their clinical translation as a nanomedicine and delivery vehicle. The current study suggests that PDEVs are mainly transported to the liver and disease sites and may be rapidly digested^{25,41}.

This paper offers a retrospective look at PDEVs from the following perspectives: Firstly, the basic properties of PDEVs, biogenesis and uptake mechanisms of PDEVs, as well as the main approaches for isolating, purifying, and anti-tumor activity; Secondly, loading methods and application of PDEVs as anti-tumor drug carriers; Thirdly, engineering strategies and applications for combinational cancer immunotherapy; Lastly, a summary of the pharmacokinetic processes of PDEVs *in vivo* was provided, along with information on the state of clinical application at the moment and the potential and difficulties of using modified PDEVs to implement cancer combination therapy.

2. Basic characteristics of PDEVs

2.1. Biogenesis and uptake process of PDEVs

Similar to MDVs, PDEVs can be assorted into three groups based on particle size and biological pathways: exosomes (with a

particle size range of 10 to 150 nm), MVs (ranging from 50 to 3000 nm), and apoptotic bodies (with a particle size range of 800 to 5000 nm)²³. Vesicles of different sizes are typically obtained using various gradient centrifugation methods. According to studies on PDEVs, the biogenesis pathways can be summarized as follows (Fig. 1A): (i) The pathway of multivesicular bodies (MVBs). This pathway is considered the primary biogenic pathway of PDEVs^{9,24,25,41}. As early as 1967, Halperin and his colleagues⁹ observed that vesicles derived from carrots were generated through this pathway. The plasma membrane fuses with MVBs, and then MVs are released into the extracellular micro-environment. Additionally, An and his workmates⁴² discovered that *Arabidopsis* derived EVs were also released through MVBs. (ii) The pathway of the exocyst-positive organelle (EXPO). MVs can be released into the extracellular space when a spherical double-membrane organelle in plant cells fuses with the plasma membrane^{43,44}. This pathway often occurs when plant cells are stressed⁴⁵. *Arabidopsis* and tobacco derived EVs are produced through this pathway^{44,46}. (iii) The vacuolar pathway. Besides the spontaneous paths of MVBs and EXPO, vacuoles can be considered a passive mode of generation. Upon plant cell pathogen invasion, vacuolar hydrolases and defense elements combine at the plasma membrane, expelling protective vesicle-derived matter extracellularly⁴⁵. The formation of central vacuoles in plant cells begins with the transformation of MVBs into smaller vacuoles that subsequently unite to generate a more prominent vacuole^{47,48}. (iv) Programmed cell death. Apoptotic bodies are produced through the normal physiological process of plant cells after orderly apoptosis and expulsion. This pathway of producing EVs is similar to that in mammals.

Limited research exists on the biogenetic mechanisms of PDEVs. The existing summaries do not adequately cover all the processes involved, and different plants may follow distinct

production pathways. Therefore, it is essential to conduct further investigations into these biogenetic mechanisms.

Although the internalization mechanism of PDEVs by recipient cells is complex and varied, five mechanisms have been proposed to explain how PDEVs are taken up (Fig. 1B). These mechanisms include membrane fusion and endocytosis^{19,49–54}. The transmembrane glycoprotein CD98 is formed by disulfide bonds connecting two chains. The heavy chain contains sugar groups such as mannose oligosaccharides, while the light chain mainly mediates amino acid transport and plays a crucial role in cellular internalization. For instance, liver cells absorb garlic derived EVs with the help of the CD98 receptor located on their surface⁴⁹. Lipids are crucial in the uptake of PDEVs, facilitating their attachment to recipient cell membranes and boosting endocytosis efficiency⁵⁰. Regente et al.⁵¹ found that macrophages took up grapefruit EVs through clathrin-dependent and micropinocytosis mechanisms. However, different PDEVs may utilize distinct uptake pathways in different recipient cells under various physiological conditions. Some specific pathways may still be unclear. Thus, further research is warranted.

2.2. Isolation methods of PDEVs

The effective isolation and enrichment of PDEVs are significant concerns in the development and utilization of PDEVs. To acquire high restoration, high purity, and low-cost PDEVs, researchers have explored various extraction and purification techniques, including ultracentrifugation, immunoaffinity separation, size exclusion chromatography, centrifugation, co-precipitation method, and electrophoresis with dialysis^{19,52,55–58}. The most popular technique for isolating PDEVs is differential ultracentrifugation. This method provides benefits, including cost-effectiveness, the removal of extensive plant tissues and

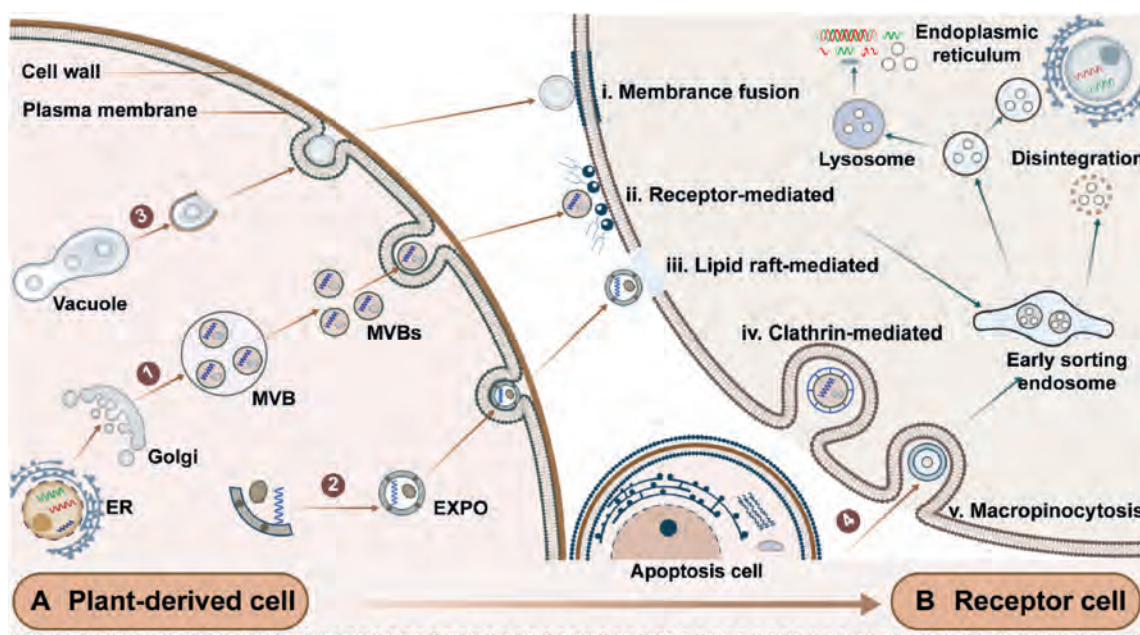


Figure 1 The formation and absorption mechanisms of PDEVs. (A) The formation process of PDEVs in plant cells: (1) Formation by vacuole, (2) Formation by MVBs, (3) Formation by EXPO, and (4) Formation by apoptotic body. (B) Absorption mechanisms in recipient cells: (i) Membrane fusion, (ii) Receptor-mediated endocytosis, (iii) Lipid raft-mediated endocytosis, (iv) Clathrin-mediated endocytosis, (v) Macropinocytosis. EXPO, exocyst positive organelle, MVBs, multivesicular bodies, ER, endoplasmic reticulum.

cellular debris, and achieving a high yield in isolation⁵⁸⁻⁶¹. The isolation method achieves the purification of PDEVs by manipulating the variation in size and density of particles⁶². Fresh plants are processed using a juice extractor and centrifugation steps with gradient-increasing speed to obtain plant liquid. Dead cells and fragments are eliminated before the sample undergoes sucrose gradient centrifugation at $150,000\times g$ ^{63,64}. Different isolation and purification methods have distinct advantages in acquiring PDEVs (Table 1)^{15,40,65-84}, and the current research direction focuses on exploring and optimizing innovative approaches to enhance the efficiency of PDEVs separation from plants. Efficient and standard extraction and separation methods are prerequisites for the exploitation and application of PDEVs; therefore, developing standardized extraction and separation technologies is essential for achieving large-scale manufacturing.

2.3. Important active ingredients and key markers of PDEVs

With the application of proteomic, lipidomic, metabolomic, and other omics technologies, the essential active ingredients of PDEVs, including nucleic acids, proteins, lipids, and metabolite compositions, have been found and studied. By promoting the intercellular communication necessary for cell growth and development, these active substances serve a critical role in preserving the physiological functions of plant cells. Additionally, they may be utilized to verify the precise molecular profiles of PDEVs from distinct species while treating a range of illnesses. In this section, we will discuss the key active ingredients and markers of PDEVs and briefly examine their anti-tumor effects and mechanisms. We listed the critical active ingredients and key markers of different PDEVs, along with their corresponding anti-tumor mechanisms that have been reported over the past decade (Table 2)^{5,15,19,40,42,63,65,80,85-108}.

2.3.1. Unique metabolite of certain PDEVs

The unique small-molecule metabolites are the primary medicinal component of PDEVs. During the biogenesis of PDEVs, the active ingredients from the medicinal plant are also preserved. Various small-molecule active ingredients have been reported, including polysaccharides, glucose, fructose, sucrose, alkaloids, glucosinates, naringin, naringenin, and others^{5,42,65,85-98}. Numerous biological actions, including anti-inflammatory, microbiome-regulating, antioxidant, anti-aging, and anticancer capabilities, are demonstrated by active compounds derived from botanical sources^{109,110}. Zeng et al.¹¹¹ successfully identified three active components (aloe-emodin, aloin, and β -sitosterol) in *Aloe vera* derived EVs using high-performance liquid chromatography. These three active ingredients have been clinically verified to have an anti-tumor and anti-inflammatory effect. Ou et al.⁹⁴ identified vinpocetine in *Catharanthus roseus* derived EVs and demonstrated its role in promoting lymphocyte proliferation in mouse models. In addition to the common anti-inflammatory and intestinal microbiota regulatory effects, we discussed the role of PDEVs in tumor treatment and their potential anti-tumor mechanisms over the past decade in Table 2. The polysaccharides, alkaloids, saponins, and other ingredients in PDEVs may have anti-tumor effects by preventing tumor cell growth, triggering tumor cell death, and reducing tumor-associated inflammation.

Identifying and analyzing more small-molecule compounds contained in PDEVs and studying their potential anti-tumor effects is of great value for the development of anti-tumor therapeutic agents based on PDEVs, which can effectively promote the

development and application of PDEVs. Nevertheless, identifying, quantifying, and utilizing these bioactive small molecules in PDEVs is a relatively new field of research. Comprehensive studies are urgently needed to elucidate the mechanisms and anti-tumor activity of the individual tiny molecules that comprise PDEVs.

2.3.2. Nucleic acids

Multitudinous research has indicated that the main nucleic acids contained in PDEVs are RNAs, and DNAs are rarely reported^{64,112}. These RNAs, enclosed in PDEVs, participate in various signaling pathways within cells and serve as mediators of intracellular communication. The RNA sequencing method has identified multiple types of functional small RNAs (sRNAs) in PDEVs. These sRNAs typically consist of 20–30 nucleotides in length. sRNAs reported include miRNAs, hcRNAs, and transfer RNAs. Nucleic acids are vital biological regulators that govern cellular proliferation, maturation, and immune defense^{112,113}. Numerous studies indicate that the types, composition, and function of sRNAs in different PDEVs vary by species^{112,113}. For example, ginger-derived EVs' RNA composition differs from that in ginger tissues. Ginger tissues have a higher tRNA content than ginger derived EVs, while ginger derived EVs contain a greater diversity and quantity of miRNAs than ginger tissues¹¹³. sRNAs in PDEVs, which serve as a means of intercellular communication, regulate various signaling pathways^{114,115}. For example, the sRNAs of ginger derived EVs can inhibit bacterial growth by downregulating the expression of gene¹¹⁶. They can also restrict the level of target genes by combining them with the three untranslated regions of recipient genes¹¹⁷.

It has been shown that miRNAs, one of these sRNAs, are essential for the possible anti-tumor effects^{19-24,63,100-104}. For example, miR167a in EVs produced from broccoli may induce apoptosis in human pancreatic cancer cells by activating the PI3K-AKT signaling pathway and upregulating genes associated with pro-apoptotic effects¹⁰⁰. Yin et al.¹⁰¹ used real-time quantitative polymerase chain reaction (RT-qPCR) and high-throughput sequencing to examine 21 miRNAs in ginger derived EVs. They discovered that by controlling inflammatory and cancer-related pathways and downregulating the expression of NF- κ B and TNF- α , Bdi-miR5179, Csi-miR396e-5p, and Ptc-miR396g-5p, among others, may prevent the formation of colon cancer cells. Yang et al.¹⁰³ found that miRNAs in bitter melon derived EVs can reverse the resistance of oral squamous cell carcinoma by hindering the activation of NLRP3 inflammation. In addition, miR159a, miR162a, miR166b, miR396b in broccoli, apple, pomegranate, orange derived EVs have potential anti-tumor value by inhibiting the proliferation of human colonic adenocarcinoma cells¹⁰⁴.

As discussed above, these sRNAs in PDEVs show promising prospects for anti-tumor applications, and further investigations are needed to determine their potential anti-tumor effects and the underlying mechanisms.

2.3.3. Proteins

Different PDEVs exhibit distinct protein profiles, which are typically influenced by the secretion pathway and mechanism of origin of PDEVs⁸⁶. In a recent study, researchers utilized proteomic methods to identify and characterize the protein composition of PDEVs. Proteolysis, heat shock, vesicular transport, chloroplast, and cell wall-associated proteins are among the prominent protein families found in PDEVs¹¹⁸. Heat shock proteins (HSPs)

Table 1 Characteristics of different extraction and separation methods of PDEVs.

Separation/purification	Advantage	Disadvantage	Application for acquiring PDEVs	Ref.
Differential centrifugation	(i) High repeatability; (ii) Large number of samples; (iii) Little effect on the sample; (iv) Simple process.	(i) Expensive cost; (ii) Time-consuming; (iii) Low purity.	(i) Strawberry-derived EVs: Ultracentrifugation under the centrifugal force of $110,000\times g$ for 1 h. (ii) Broccoli-derived EVs: Ultracentrifugation under the centrifugal force of $100,000\times g$ for 1.5 h. (iii) Sunflower-derived EVs: Centrifugation under the centrifugal force of $40,000\times g$ for 1 h and ultracentrifugation under the condition of $100,000\times g$ for 1 h. (iv) Nicotiana benthamiana-derived EVs: Centrifugation under the centrifugal force of $10,000\times g$ for 0.5 h and ultracentrifugation under the centrifugal force of $100,000\times g$ for 3 h. (v) <i>Arabidopsis</i> -derived EVs: Centrifuged sequentially at $700\times g$ for 10 min, $2000\times g$ for 20 min, and $10,000\times g$ for 30 min. Ultracentrifugation followed at $100,000\times g$ for 3 h. (vi) <i>Flos sophorae immaturus</i> -derived EVs: Centrifugation under $100,000\times g$ for 70 min. (vii) Grapefruit-derived EVs: Differential centrifugation under the $2000\times g$ for 20 min, $15,000\times g$ for 0.5 h, and $110,000\times g$ for 1.5 h; differential centrifugation under the $1500\times g$ for 0.5 h, $10,000\times g$ for 1 h, and $16,000\times g$ for 60 min, and 10,000 overnight. (viii) Ginseng-derived EVs: Differential centrifugation under the $3000\times g$ for 0.5 h, $10,000\times g$ for 1 h, and $150,000\times g$ for 2 h. (ix) Shiitake Mushroom-derived EVs: Differential centrifugation under $200\times g$ for 20 min, $10,000\times g$ for 0.5 h, and $100,000\times g$ for 2 h.	15,65-73

(continued on next page)

Table 1 (continued)

Separation/purification	Advantage	Disadvantage	Application for acquiring PDEVs	Ref.
Density gradient centrifugation	(i) High purity; (ii) High separation efficiency; (iii) Extensive use; (iv) PDEVs structure preserved.	(i) Expensive cost; (ii) Time-consuming; (iii) Complicated operation; (iv) Low yield.	(i) Ginseng-derived EVs: Sucrose gradient of 8%, 30%, 45% and 60% under 150,000×g for 2 h. (ii) Ginseng-derived EVs: Sucrose gradient of 15%, 30%, 45%, and 60% under 150,000×g for 1 h. (iii) Ginger-derived EVs: Sucrose gradient under 100,000×g for 16 h. (iv) Ginger-derived EVs: Sucrose gradient of 8%, 30%, 45%, and 60% under the condition of 150,000×g for 2 h. (v) <i>Arabidopsis</i> -derived EVs: Iodixanol gradient of 5%, 10%, 20%, and 40% under the condition of 100,000×g for 117 h. (vi) Grapefruit and turmeric-derived EVs. (i) <i>Arabidopsis</i> -derived EVs: Incubation under the condition of specific antibody.	40,74-79
Immunoaffinity capture method	(i) High specificity; (ii) High purity; (iii) Low cost.	(i) Not suitable for complex ingredients; (ii) Low yield; (iii) Absence of standardized PDEVs indicators; (iv) Insufficient availability of commercially produced antibodies.		80-82
Polymer precipitation method	(i) Simple operation; (ii) Low cost; (iii) The structure of PDEVs remains intact.	(i) Co-precipitation of other biological pollutants; (ii) Low purity.	(i) Cabbage-derived EVs: Coprecipitation under the condition of 20% PEG for 24 h and centrifugation under 1500×g for 0.5 h.	82,83
Size exclusion chromatography	(i) Good separation effect; (ii) High purity; (iii) The structure of PDEVs remains intact.	(i) Low capacity; (ii) Special equipment and techniques are required;	(i) Cabbage and ginseng-derived EVs.	83
Ultrafiltration	(i) Easy and fast execution; (ii) High recovery rate; (iii) Low cost.	(i) Membrane blockage; (ii) Particle loss; (iii) Low purity.	(i) Blueberry and <i>Arabidopsis</i> -derived EVs: Ultrafiltration under different molecular weights.	84

Table 2 The essential active ingredients, anti-tumor effects, and key markers of PDEVs.

Biochemical profiles	PDEVs	Components or markers	Potential anti-tumor functions and mechanisms	Ref.
Unique metabolite of certain PDEVs	Broccoli-derived EVs	(i) Sulforaphane.	(i) Inhibit tumor growth, metastasis and angiogenesis by impacting acetylation of histones and modifying intracellular signaling.	65,85
	Ginger-derived EVs	(i) 6-Gingerol, 6-shogaol.	(i) Reduce the stemness of breast cancer stem cells by influencing hedgehog/Akt/GSK3 β signaling.	5,86,87
	Lemon-derived EVs	(i) Polysaccharides, citric acid.	(i) Inhibit tumor development and modulate the levels of immune cells.	88,89
	Oat-derived EVs	(i) β -Glucan.	(i) Inhibit the activation of tumor-related inflammatory signals.	42,90
	Ginseng-derived EVs	(i) Ginsenosides.	(i) Improve immune response, reverse medication resistance to chemotherapy, and trigger tumor death <i>via</i> modulating signaling pathways of MAPK, PI3K/Akt/mTOR.	91,92
	Orange-derived EVs	(i) Glucose, fructose, sucrose.	(i) Not reported.	93
	<i>Catharanthus roseus</i> -derived EVs	(i) Vinpocetine, alkaloids.	(i) Reduce the amounts of tumor-promoting inflammatory factors, including IL-2, and IL-6.	94,95
	Grapefruit-derived EVs	(i) Naringin, naringenin.	(i) Block the tumor cell cycle, reduce tumor cell growth, promote tumor cell death, overcome tumor cell drug resistance, and improve chemotherapeutic drug sensitivity.	96-98
	Cucumber	(i) Cucurbitacin B.	(i) Reduce the growth of human non-small cell lung cancer cells by inhibiting STAT3 activity.	99
	Broccoli-derived EVs	(i) Bna-miR167a_R-2.	(i) Regulate the PI3K-AKT signaling pathway in pancreatic cancer cells and promote tumor cell death.	100
Nucleic acids	Ginger-derived EVs	(i) Bdi-miR5179. (ii) Csi-miR396e-5p. (iii) Ptc-miR396g-5p. (i) miR166 family.	(i) Regulate immune response and tumor-related inflammation by lowering levels of NF- κ B, IL-6, IL-8, and TNF- α .	101
	Blueberry-derived EVs	(i) miRNAs.	(i) Not reported.	102
	Bitter melon-derived EVs		(i) Reverse the resistance of oral squamous cell carcinoma by hindering the activation of NLRP3 inflammation.	103
	Soybean-derived EVs	(i) miR-5781.	(i) Inhibit tumor-related inflammatory responses.	63
	Broccoli, apple, pomegranate, orange-	(i) miR159a, miR162a, (ii) miR166b, miR396b.	(i) Inhibit the proliferation of human colonic adenocarcinoma cells.	104
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Table 2 (continued)

Biochemical profiles	PDEVs	Components or markers	Potential anti-tumor functions and mechanisms	Ref.
Lipids	derived EVs	(i) Ceramide.	(i) Transform tumor-associated macrophages from M2 to M1 polarization.	40
	Ginseng-derived EVs	(ii) Phosphatidylethanolamine.	(i) Not reported.	105
	Ginger-derived EVs	(i) Digalactosyl-diacylglycerol.	(i) Not reported.	19
		(ii) Monogalactosyl, monoacylglycerol.	(i) Not reported.	19
Proteins	<i>Arabidopsis</i> -derived EVs	(i) Sphingolipids.	(i) Not reported.	106
	<i>Arabidopsis</i> -derived EVs	(i) Potential markers of PEN1, PEN3 and TET8; Regulate cell differentiation, transport sRNA.	(i) Not reported.	80,107
	<i>Salvia dominica</i> hairy root-derived EVs	(i) Potential markers of TET7.	(i) Not reported.	108
	Sunflower-derived EVs	(i) Potential markers of Rab11; Mediates the fusion of vesicle and receptor cell membranes.	(i) Not reported.	15
	Ginseng-derived EVs	(i) Surface protein.	(i) Promote the polarization of M1 macrophages.	40

MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; mTOR, mechanistic target of rapamycin; IL-2, interleukin-2; IL-6, interleukin-6; STAT3, signal transducer and activator of transcription 3; AKT, protein kinase B; NF- κ B, nuclear factor kappa-B; IL-8, interleukin-8; TNF-2, tumor necrosis factor receptor-2; PEN1, penetration1; PEN3, penetration3; TET8, tetraspanin 8; TET7, tetraspanin 7.

are a prevalent class of proteins in PDEVs. HSP60, HSP70, HSP80, and HSP90 proteins have been identified in the *Arabidopsis thaliana*, olive leaf, tobacco, sunflower, bitter melon, and tomato derived EVs. HSPs are reactive proteins that can withstand stress and disturbance when Chinese herbs are exposed to high temperatures¹¹⁹. These proteins are essential for plant growth, nutrient absorption, and cell development.

Two other abundant protein families in PDEVs are annexins and aquaporins. Membrane proteins are distributed on the surface of the lipid bilayer and are primarily responsible for vesicle transport and the regulation of ROS. Several membrane proteins, including annexin 1 and P34, have been identified in *Arabidopsis* and sunflower-derived EVs^{51,120,121}. Aquaporin is responsible for moving water and gases^{42,122}. Recent research has shown that these vesicles store defense and resistance proteins, playing a role in regulating key signaling of PDEVs. For instance, antifungal proteins powdery mildew resistant 5 (PMR5) and (ginkbilobin-2) GNK2, as well as RPM1 interacting proteins, have been found in *Arabidopsis* and sunflower derived EVs^{51,123}.

In addition to the common proteins mentioned earlier, PDEVs contain a range of proteins with potential anti-tumor properties. For instance, the surface protein II lectins found in garlic derived EVs can attach to the CD98 glycoprotein on the target cells¹²³. This indicates that targeted delivery vectors for anti-tumor drugs can be designed under the action of surface protein II lectins in garlic derived EVs to increase the accumulation at tumor sites. Furthermore, proteins from ginseng derived EVs may induce the polarization of M2 macrophages into M1 macrophages, contribute to the modulation of the immunological microenvironment mediated by ginseng-derived EVs, and limit the development of melanoma⁴⁰.

2.3.4. Lipids

The basic components of PDEVs, lipids, are essential for preserving the biological functions and structural stability of PDEVs¹²⁴. In recent years, researchers have conducted lipid-omics analysis of PDEVs primarily using a liquid chroma-tograph mass spectrometer^{16,124}. Current studies have revealed that the main lipid classes in PDEVs can be categorized as glycolipids and phospholipids. The lipids identified in PDEVs include phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidic acid (PA)¹²⁵. The lipids in PDEVs are mainly utilized for treating inflammation-related diseases and engineered as drug carriers. Lipids in ginger derived EVs can alleviate obesity caused by a high-fat diet and promote intestinal digestion and absorption by upregulating Foxa2 expressed by the intestinal epithelium¹²⁶. It can also alleviate periodontitis caused by *Porphyromonas gingivalis*^{78,126}.

In addition to being used for inflammation-related diseases, the lipids in PDEVs also have potential anti-tumor applications. Cao et al.⁴⁰ utilized lipid-omics technology to investigate the lipid components responsible for macrophage polarization in ginseng derived EVs. This work reveals that digalactosyl mono-acylglycerol, PE, and ceramide are involved in inducing tumor-associated macrophages to switch from the M2 phenotype to the M1 phenotype via a TLR4-MyD88-dependent process.

Although numerous researchers have discovered the significant role of lipid components, our understanding of PDEVs is still insufficient. Consequently, a multi-technological approach is essential for precise lipid characterization within PDEVs, enabling the exploration of potential anti-tumor components and mechanisms of PDEVs and promoting the development of anti-tumor therapeutic agents based on PDEVs.

2.3.5. Key markers of different PDEVs

Key indicators of PDEVs are chemicals that are present in the majority of PDEVs and may particularly reflect the location, cell origin, and secretion mechanism of PDEVs. Due to the heterogeneity of specific small molecule substances and nucleic acids in PDEVs, the identification and study of potential key markers of PDEVs typically focus on protein and lipid components^{42,122}. Several protein families have been identified in PDEVs, including HSPs, penetration 1 (PEN1), and Tetraspanin-8 (TET-8)^{42,122}. HSPs are widely recognized in PDEVs and reside in the membrane of vesicles, playing a crucial role in maintaining the integrity of the vesicular structure¹¹⁸. Meyer et al.¹²⁷ and He et al.¹²⁸ found that PEN1 co-localized with the amphiphilic styrene dye FM4-64 outside the plasma membrane, whereas TET-8 co-localized with the MVB markers in plant cells. This indicates that it may serve as a marker for PDEVs. Recent research has shown that different PDEVs possess similar types of lipids, with the major ones being PA, phosphatidylcholine PC, and PE, indicating that they may act as key markers for PDEVs.

Currently, research on the essential indicators of PDEVs is in its early phases. Few studies have identified the essential indicators of PDEVs. Research on the leading indicators of PDEVs faces significant hurdles due to the effect of elements such as species, part, season, growing environment, extraction, separation, and purification procedures on the source of PDEVs. With the advancement of technology, such as artificial intelligence, research on critical indicators of PDEVs is expected to make significant advances in the future.

Contemporary research identifying and analyzing components of PDEVs mainly focuses on the profiles of unique metabolites, nucleic acids, proteins, and lipids. Compared to MDV's identification components, the identification components for PDEVs are still in the early stages. Establishing a standardized PDEVs component identification is urgently needed and can be generally applied to different plant species.

2.4. Anti-tumor activity of PDEVs

Because of tumor drug resistance and drug-related adverse reactions, formulating novel nanomedicines remains a significant challenge for scientists^{129,130}. Several natural compounds from Chinese herbal medicines have emerged as potential alternative strategies for tumor prevention and treatment. Numerous studies highlight the tumor-suppressing potential of PDEVs, attributed to their safe origin, minimal side effects, nanoscale size, and potent anti-tumor activity (Fig. 2)^{47,113,114,131-133}. PDEVs offer promise in cancer treatment by prompting tumor cell death, modulating inflammatory agents, and altering the tumor's surroundings^{59,113,134,135}. The anti-tumor mechanism of PDEVs in various tumors is summarized in Table 3^{16,40,53,58,59,75,91,94,100,101,103,113,115,134,136-161}.

A previous study discovered that tea leaves derived from EVs could effectively suppress breast tumor cell growth by increasing ROS production¹⁴⁵. Elevated reactive oxygen species levels, triggering mitochondrial harm and G1/S phase cell cycle cessation, consequently prompted malignant breast cells' programmed demise. Chen et al.⁵⁸ utilized differential centrifugation to prepare tea flower derived EVs and used them to load polyphenols and flavonoids to treat breast cancer. In models of mouse breast cancer, tea flower derived EVs carrying drug-active compounds may prevent lung metastases and tumor development. This is achieved by inducing apoptosis in cancer cells and halting the cell cycle at

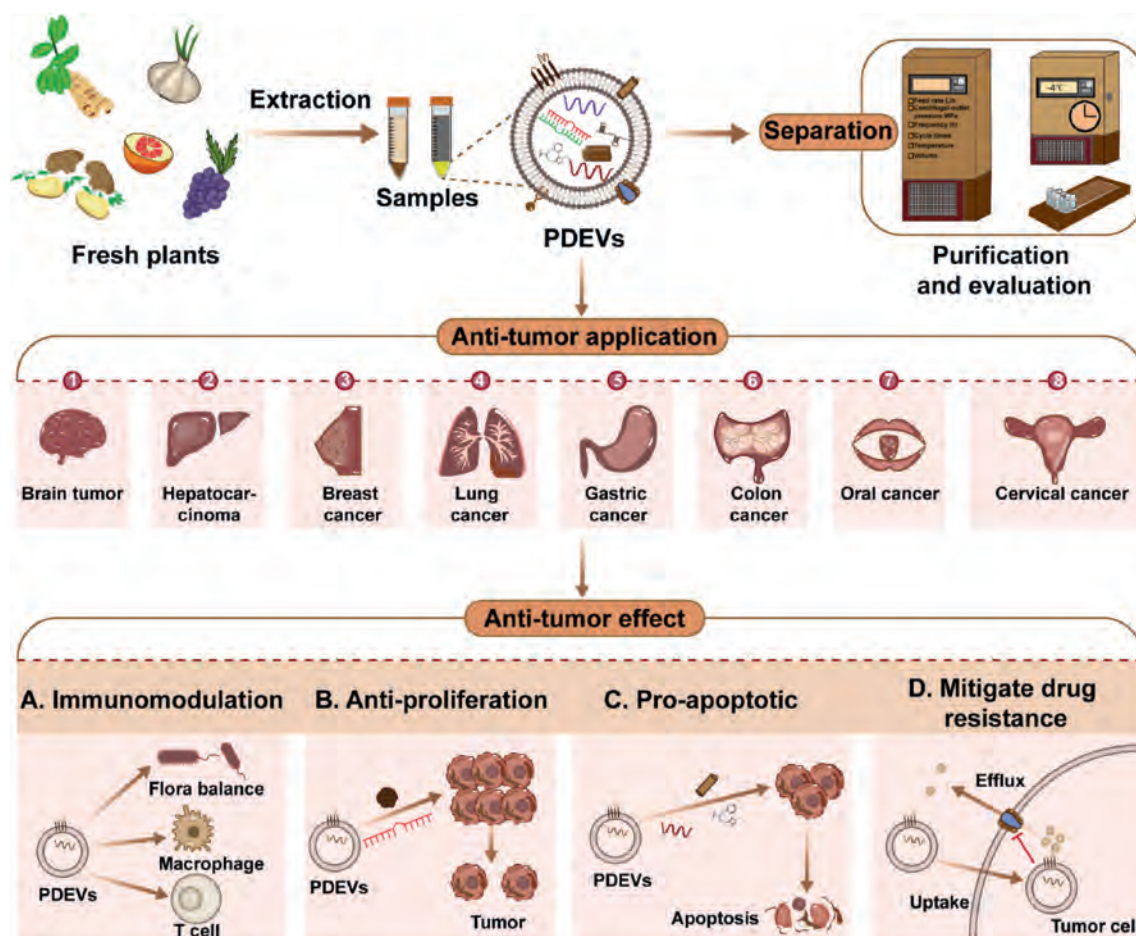


Figure 2 Extraction, isolation, and anti-tumor applications of PDEVs, including their notable anti-tumor effects.

the G2/M phase. Additionally, these EVs can lead to mitochondrial damage in tumor cells by increasing reactive ROS levels. Compared to the control group, plant vesicles can inhibit the formation of pulmonary nodules.

Kim et al.⁹¹ found that ginseng-derived EVs caused apoptosis in C6 glioma cells by boosting macrophage proliferation and altering the immunological milieu. Their study revealed that the primary mechanisms through which ginseng derived EVs suppressed cancer cells were by promoting the formation of BAX, downregulating anti-apoptotic genes, inhibiting the conversion of M0 macrophages to M2 macrophages, and decreasing the production of pro-tumoral cytokines. Ginseng derived EVs regulated the tumor microenvironment by reducing iNOS, EGF, VEGF, and TGF- β expression, thus suppressing neoplastic proliferation. Furthermore, ginseng-derived EVs could shift the polarization of macrophages, eliminating cancer cells. It also exhibits a good permeation effect on solid tumors and safe distribution characteristics in major organs. Cao et al.⁴⁰ found that ginseng-derived EVs triggered the toll-like receptor 4 signaling pathway, repolarizing the M2 to M1 phenotype. This alteration in macrophages may contribute to the production of ROS, which effectively kills tumor cells. Similarly, *Artemisia annua* L. derived EVs can also influence the phenotype of macrophages, which could improve the level of M1 phenotype by increasing the production of ROS¹⁶⁰.

In addition to the PDEVs mentioned above, garlic and *Asparagus cochinchinensis* exhibit anti-tumor activity^{16,149}. Garlic derived EVs have been shown to curb kidney and lung cancer cell

proliferation effectively. These EVs exert their impact by putting the brakes on the cell cycle right in the S phase, ratcheting up the formation of reactive oxygen species, and putting a damper on the genes and proteins that normally prevent cancer cells from dying¹⁵¹. EVs from *Asparagus cochinchinensis* Merr suppress the growth of human liver cancer cells by inducing programmed cell death and increasing the presence of proteins connected to apoptosis¹⁶.

3. PDEVs as delivery vehicles

3.1. Loading techniques

Compared to synthetic nanoparticles, PDEVs offer several benefits as drug delivery carriers, including minimal immunogenicity, efficient absorption rates, specialized targeting capabilities, and high stability^{5,47}. PDEVs are nanoscale particles that exhibit notable tissue permeability, a negative surface charge, a plasma membrane-like composition, and robust stability^{23,162}. Additionally, PDEVs have excellent tumor-targeting and tissue-penetrating abilities, allowing them to cross the blood-brain barrier effectively¹³¹. Loading drugs in PDEVs can prevent the breakdown of proteases and nucleases.

While PDEVs are an excellent drug delivery carrier, selecting an appropriate loading method is crucial for preserving drug integrity and achieving high drug delivery efficiency¹⁶³⁻¹⁶⁶. A suitable approach not only enables high drug encapsulation but

Table 3 The anti-tumor mechanism of PDEVs.

PDEV	Mechanism and pathway of action	Ref.
Ginger-derived EVs	(i) Regulate tumor-related inflammation, reduce pro-inflammatory cytokines like interleukin-6, interleukin-1 β , and tumor necrosis factor- α , raise anti-inflammatory cytokines like interleukin-10 and interleukin-22, and promote heme oxygenase-1. (ii) Breast cancer: Reduces ROS formation, causes mitochondrial damage, and stops the cell cycle in the G2/M phase.	75,101,113,136-139
Ginseng-derived EVs	(i) Melanoma: Stimulates toll-like receptor 4 and MyD88 signaling and promotes M2 macrophages to M1 macrophages; enhances T lymphocyte infiltration; turns cool tumors into hot tumors. (ii) Lung cancer: Inhibit glycolysis of tumor cells by downregulating the pentose phosphate pathway (iii) Colorectal cancer: Increase the level of C-C motif chemokine ligand 5 and decreases the level of immune checkpoint expression of T cells. (iv) Glioma: Suppresses tumor-associated macrophage growth and reduces c-MYC gene expression. (v) Other tumors: Increase the activity of tumor-infiltrated T lymphocytes;	40,91,140-142
Grapefruit-derived EVs	(i) Brain tumor: Inhibits the growth of brain tumors. (ii) Melanoma: Arrests cell cycle at G2/M. (iii) Colon cancer: Promotes the polarization of M1 macrophages. (iv) Inflammation regulation: Inhibits the production of proinflammatory factors.	75,134,143
<i>Asparagus cochinchinensis</i> (Lour.) Merr.-derived EVs	(i) Hepatocellular carcinoma: Inhibits the growth of tumors; activates caspase-9 and induces cell apoptosis.	16
Bitter melon-derived EVs	(i) Oral squamous cell carcinoma: Impede the action of the inflammasome; combined with 5-FU to increase the generation of ROS and thus enhance the apoptosis of oral squamous cell carcinoma cells; down-regulating the expression of the inflammasome NLRP3 enhances the cytotoxic effect of 5-FU during the treatment of oral squamous cell carcinoma and reduces drug resistance.	103
Tea leaf-derived EVs	(i) Breast tumor: anti-proliferation and pro-apoptosis <i>via</i> the cellular signal pathways associated with the cytokine-cytokine receptor interaction, JAK-STAT, and cell cycle. (ii) Colitis-associated colon cancer: Causes mitochondrial damage in tumor cells, stops the cell cycle, and triggers cancer cell death.	100,145
Tea flower-derived EVs	(i) Metastatic breast cancer: Causes mitochondrial damage in tumor cells, stops the cell cycle, and triggers cancer cell death.	58
Turmeric-derived EVs	(i) Oral squamous cell carcinoma: act as collaborative synergists to surmount bacteria-induced drug resistance for enhanced chemotherapy.	144
Broccoli-derived EVs	(i) Human pancreatic cancer cells: Regulate the PI3K-AKT signaling pathway and cause apoptosis in pancreatic cancer. (ii) Colon cancers: Inhibit HT-29 cell proliferation and migration; increase HT-29 cell death; reverse chemoresistance to 5-FU in HT-29 cells by reducing aberrant activation of the PI3K-Akt-mTOR signaling pathway.	146,147
Corn-derived EVs	(i) Colon26 tumor cells: Inhibit cell proliferation; increase production of tumor necrosis factor α .	53
<i>Dendropanax morbifera</i> -derived EVs	(i) Melanoma: Downregulates the content of melanin; inhibits the activity of tyrosinase.	148
Garlic-derived EVs	(i) Inhibit cGAS-STING-mediated inflammatory responses. (ii) Kidney cancer: Inhibit cell proliferation. (iii) Renal cancer: Increase the gene expression levels of pro-apoptotic genes and decrease the gene expression levels of anti-apoptotic genes, therefore increasing cancer cell death.	149-151
<i>Morus nigra</i> L. leaves-derived EVs	(i) Hepatocellular carcinoma: Improves oxidative stress, induces damage to mitochondria, and inhibits the proliferation of tumor cells.	152

(continued on next page)

Table 3 (continued)

PDEV	Mechanism and pathway of action	Ref.
<i>Lonicera japonica</i> Thunb.-derived EVs	(i) Cervical cancer: Induces cell apoptosis by MiR2911 bound to HPV16/18 E6 and E7 oncogenes.	153
<i>Moringa oleifera</i> Lam-derived EVs	(i) Leukemia, cervical cancer: Reduce the creation of anti-apoptotic proteins and boost the development of pro-apoptotic proteins.	154
Rice bran-derived EVs	(i) Colorectal cancer: Arrest cell cycle; induce cell apoptosis.	155
<i>Brucea javanica</i> -derived EVs	(i) Triple-negative breast cancer: Activate the signaling pathway of PI3K–Akt; induce cell apoptosis.	115
<i>Pueraria lobata</i> -derived EVs	(i) Lipopolysaccharides-induced inflammation: Decreases the level of interleukin-6 and promotes the maturation of DCs.	156
<i>Catharanthus roseus</i> -derived EVs	(i) Increase the activation of immunological cells.	94
<i>Atractylodes lancea</i> -derived EVs	(i) Melanoma: Inhibit melanogenesis.	157
<i>Aster yomena</i> -derived EVs	(i) Promote the maturation of antigen-presenting cells.	158
<i>Petasites japonicus</i> -derived EVs	(i) Promote the maturation of DCs	159
<i>Artemisia annua</i> -derived EVs	(i) Lung cancer: Activate inactive immune cells	160
Lemon-derived EVs	(i) Gastric cancer: Up-regulate the expression of GADD45A gene and protein in three gastric cancer cell lines; stimulate ROS formation; cause gastric cancer cell cycle S-phase arrest; and induce cell apoptosis. (ii) P53-inactivated colorectal cancer: Stimulates the process of micropinocytosis and suppresses tumor cell growth.	59,161
Cucumber-derived EVs	(i) Non-small cell lung cancer: Decrease the growth of human non-small cell lung cancer cells by reducing signal transducer and activator of transcription 3 activation, causing ROS formation, inducing cell cycle arrest, and activating the caspase pathway.	99

ROS, reactive oxygen species; MyD88, myeloid differentiation primary response protein 88; mTOR, mechanistic target of rapamycin; c-MYC, myelocytomatosis viral oncogene homolog; NLRP3, nod-like receptor thermal protein domain associated protein 3; JAK, janus kinase; STAT, signal transducer and activator of transcription; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; HPV16/18, human papillomavirus 16/18; GADD45A, gene-growth arrest and DNA damage inducible alpha.

also ensures the integrity of PDEVs and the activity of drugs. The primary loading methods are co-incubation, electroporation, freeze-thaw, sonication, and liposome membrane fusion^{5,163}. Furthermore (Table 4)^{167–188}, and (Fig. 3) summarize the methods, applicability, advantages, determination, and main operating conditions for drug loading in PDEVs.

3.1.1. Electroporation

Electroporation has been extensively utilized for drug loading in EVs from mammalian¹⁸⁹. This technique achieves drug loading by applying an external electric field through a brief high-voltage pulse, forming numerous tiny hydrophilic pores on the surface of the PDEV's membrane¹⁹⁰. Once these pores are formed, the desired cargo, such as DNA, RNA, and drugs, can enter the PDEVs. Afterward, the vesicle membrane rapidly reforms itself¹⁷¹. This approach provides the benefits of ease and significant efficiency.

3.1.2. Co-incubation

Co-incubation is the most prevalent technique for incorporating drugs into PDEVs. The drug and PDEVs are incubated under appropriate conditions, allowing the drug to be encapsulated within PDEVs through diffusion and electrostatic interactions. This method is suitable for hydrophobic medicines¹⁹¹.

3.1.3. Ultrasound

The ultrasound method is similar to electroporation. In this method, the drug and PDEVs are mixed and placed into ultrasonic

equipment. Then, under specific conditions, sonication is performed using a probe. During co-sonication, the drugs diffuse into the PDEVs when the membrane undergoes deformation. It should be noted that during the sonication process, the drug is encapsulated within the PDEVs and coated on the surface of the membrane^{178,191–193}. For instance, Li et al.¹⁹⁴ utilized sonication to load astaxanthin to broccoli derived EVs. The results showed that the bioavailability of astaxanthin was improved and effectively inhibited enteritis.

3.1.4. Others

In addition to the drug mentioned above, various delivery technologies, including the freeze-thaw method, co-extrusion, freeze melting, and click chemistry, are effective loading methods. The freeze-thaw approach is widely employed for liposome membrane fusion, and the target medication is encapsulated into PDEVs through multiple freeze-thaw cycles^{177,186,188,195}. Compared to traditional cross-linking reactions, the click chemistry method displays higher efficiency in loading drug molecules onto the PDEV's surface *via* covalent bonds without influencing the PDEV's structure and function^{5,188,196}.

3.2. PDEVs as delivery vehicles for anti-tumor drugs

Anti-tumor drugs have poor targeting ability and cannot accurately act on tumor cells. While killing cancer cells, they will also harm healthy cells and tissues, which will have detrimental

Table 4 The main advantages and disadvantages of loading strategies for PDEVs.

Loading strategy	Process and operational condition	Applicable type	Merit	Shortcoming and limitation	Ref.
Electroporation	Through the effect of a high-intensity external electric field, small holes are instantly formed, increasing the permeability of the PDEVs' membrane and enabling the drug to be loaded into the PDEVs.	Mainly suitable for nucleic acid drugs.	(i) The drug loading process is simple and can be achieved under laboratory conditions. (ii) The permeability of the PDEV membrane can be effectively improved in a short period. (iii) The operation time is short, and it can load drugs quickly. (iv) The operation method is simple and convenient.	(i) It is prone to cause the accumulation and agglomeration of nucleic acid substances. (ii) The drug loading efficiency is relatively low. (iii) Cannot be widely applied to various types of drugs.	167-171
Co-incubation	It belongs to the passive drug loading strategy, mainly loading drugs into PDEVs under hydrophobic interactions, passive diffusion, and electrostatic interactions.	Small-molecule hydrophobic drug molecules.	(i) Easy to operate under laboratory conditions. (ii) The drug loading process is mild. (iii) The drug loading process will not have an impact on the drug.	(i) The drug loading efficiency usually depends on specific incubation conditions, such as appropriate time, temperature, and pH value. (ii) In the absence of other external forces to promote drug diffusion, the drug loading efficiency is usually low.	172-174
Sonication	An active loading strategy. Under the action of ultrasonic waves, mechanical and thermal effects cause drugs and PDEVs to vibrate at a specific frequency, thereby loading drug molecules into the PDEVs.	Hydrophilic drug molecules or macromolecular proteins.	(i) High drug loading efficiency. (ii) Uniform particle size.	(i) Damage the integrity of the PDEV's structure. (ii) Prone to cause the leakage of drugs. (iii) Not suitable for heat-unstable drugs.	175-179
Liposome membrane fusion method	The membrane fusion between the drug-loaded liposomes and the PDEV membrane was induced using the PEG induction method, the ultrasound method, and the repeated freeze-thaw method, after which the drug was loaded into the PDEVs.	Suitable for both fat-soluble and hydrophilic drugs.	(i) Increase the drug loading capacity. (ii) Maintain the integrity of the structure and functions of PDEVs. (iii) Uniform particle size.	(i) Compared with other drug loading methods, it is more complex and has a higher cost.	180-184

(continued on next page)

Table 4 (continued)

Loading strategy	Process and operational condition	Applicable type	Merit	Shortcoming and limitation	Ref.
Co-extrusion	This method involves repeatedly passing the drug and PDEVs through a filter with a specific pore size. While encapsulating the drug into the PDEVs, nanoparticles with uniform particle size are obtained.	Suitable for both fat-soluble and hydrophilic drugs.	(i) High drug loading efficiency. (ii) The operation method is simple and convenient. (iii) The particle size is uniform and controllable.	(i) The integrity of the PDEV structure will be damaged during the extrusion process. (ii) It is not suitable to load two or more drugs simultaneously because it is difficult to control the proportion of the drugs during the extrusion process.	173,175,176,185
Osmotic shock method	By changing the osmotic pressure of PDEVs over a short period, the PDEV membrane becomes slightly cracked, allowing drug molecules to enter through osmotic pressure.	Macromolecular proteins, hydrophilic drugs.	(i) High drug loading efficiency. (ii) The operation method is simple. (iii) The size of PDEVs and the zeta potential can be better retained. (iv) It does not affect the biological activity of PDEVs.	(i) The surface membrane protein of the PDEV structure may be damaged.	186
Freeze melting method	Through rapid low-temperature freezing, ice crystals form inside PDEV, causing the PDEV membrane to rupture. During the melting process, the PDEV membrane regains its integrity, and simultaneously, the drug penetrates the PDEVs. Connect drug molecules to the surface of PDEVs through chemical reactions.	Chemical compounds	(i) Maintain the integrity of the PDEV's structure. (ii) High drug loading efficiency. (iii) Uniform particle size.	(i) PDEVs are prone to aggregation during the drug loading process.	187
Click chemistry		Chemical compounds	(i) High drug loading efficiency.	(i) The drug loading process is greatly influenced by the environment.	188

PEG, Polyethylene glycol.

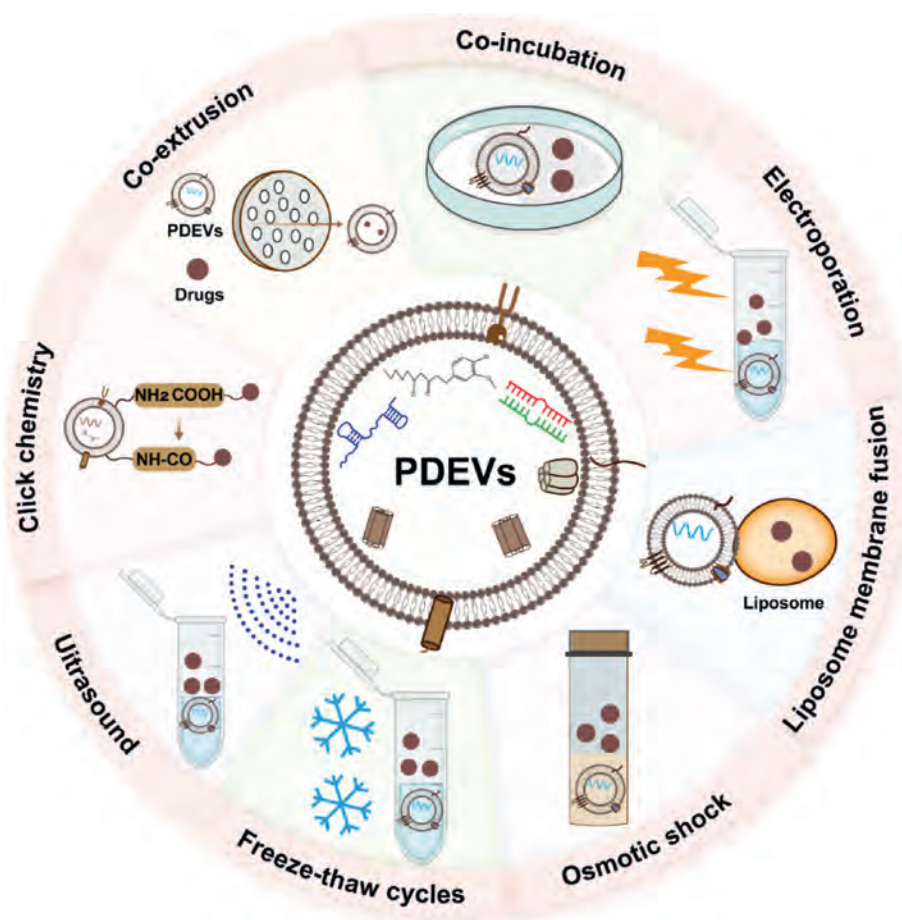


Figure 3 The loading methods of PDEVs.

repercussions, which is a significant pain point in current treatment. Research indicates that plant vesicle-based drug delivery systems exhibit low immunogenicity, enhanced cellular uptake, and considerable stability *in vivo*⁵. Plant vesicles exhibit good biocompatibility, weak immune response after entering the body, low toxicity, and high safety, which may decrease the harmful effects and side effects of cancer medication^{71,197}. Notably, in the tumor microenvironment, PDEVs are attributed to specific molecular markers on their surface, enabling them to identify and bind the corresponding receptors on the tumor cell surface, thus achieving accurate drug delivery¹⁹⁸. Hence, PDEVs have proven effective carriers for diverse anti-cancer therapeutics, encompassing small molecules and nucleic acids, in real-world scenarios¹³¹.

Ginger derived EVs with target ability by binding to folate and loaded with doxorubicin to treat intestinal cancer cells. Compared to free drugs, the modified EVs exhibited better effects¹⁹⁹. Simultaneously, it exhibits superior pH-responsive drug release compared to liposomal doxorubicin (DOX)¹⁷⁵. Huang et al.²⁰⁰ found that edible and cationic-free kiwifruit-derived vesicles could mediate EGFR-targeted siRNA delivery, thereby inhibiting multidrug-resistant lung cancer. Besides, Yan et al.¹¹⁵ isolated exosome-like nanovesicles derived from Traditional Chinese medicine for the first time and found that they could serve as an efficient active miRNA delivery nanoplatform, inhibiting breast cancer growth, metastasis, and angiogenesis, which has excellent potential as a new biotherapeutic treatment

for triple-negative breast cancer. Meanwhile, Liu et al.¹⁶⁰ found that mitochondria DNA extracted from medicinal plants could remodel tumor-associated macrophages and promote tumor regression by inducing the cGAS–STING pathway through nanoparticles.

PDEVs have promising applications as emerging nanocarriers for cancer therapy (Table 5)^{31,58,83,103,111,115,116,126,146,148,175,181,194,200-210}. With the continuous deepening of related research and technological progress, these developments are expected to bring more efficient and safe treatment strategies for cancer.

4. Engineering strategies for cancer therapy

PDEVs have a substantial internal capacity, making them suitable carriers for loading diverse active substances in drug delivery. However, due to variations in species sources, PDEVs exhibit limitations in effectively targeting mammalian cells²¹¹. Currently, the *in vivo* localization of most PDEVs primarily depends on passive targeting, which often results in low bioavailability. To improve drug targeting abilities and enhance effective accumulation at the treatment site while minimizing toxicity to normal tissues, the surface engineering technology of PDEVs has attracted considerable attention. In this section, we will explore the targeted modification strategy of PDEVs and examine the possibility of active targeting (Table 6)^{16,31,32,143,175,181,200,203,207,210-222} and (Fig. 4).

4.1. Innate tumor targeting of PDEVs

PDEVs are naturally able to target tumors. PDEVs from various sources may use surface adhesion proteins to target recipient cells specifically. Additionally, PDEVs are highly biocompatible and have a tiny particle size. Innate targeting may be achieved by utilizing the increased permeability and retention effect at the tumor site to keep them there. Ginsenoside Rg3, for example, is rich in ginsenoside EVs, which may bind to glucose-transporting protein 1 (GLUT1), be abundantly produced on the tumor surface, and facilitate tumor targeting^{40,142,223}. Likewise, Kilasniya et al.⁷¹ synthesized tomato and grapefruit derived EVs and assessed their glioma-targeting and antioxidant properties. Their findings have shown that HSP70 can be efficiently delivered to glioma cells *via* EVs derived from grapefruit and tomato. This study demonstrated the innate brain tumor-targeting ability of EVs derived from grapefruit and tomato; however, the specific targeting mechanism requires further in-depth research.

4.2. Physical modification

Physical modification techniques include light, heat, magnetism, electricity, and ultrasound. Incorporating photosensitizers, thermosensitizers, sonosensitizers, or other stimuli-responsive materials into PDEVs makes it possible to achieve precise delivery of drugs^{224,225}. For example, light-responsive delivery platforms can precisely control drug release by adjusting parameters such as wavelength, power, and irradiation time^{226,227}.

The membrane of PDEVs is made of lipid bilayers. Lipid or lipid-labeled molecules can integrate into membranes *via* hydrophobic forces, which help to functionalize the surface of PDEVs²²⁸. In various studies, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG) has been employed as a linker between PEDVs and targeted ligands. Specifically, DSPE is hydrophobically inserted into the lipid bilayers of PDEVs to anchor the membrane while the ligand is attached to the other side of the PEG. For instance, Feng et al.³² utilized DSPE -(PEG)2000-MAL to attach the HA binding targeting fragment to the grapefruit derived EV surface. This modification enabled the TP-EVS-STs (ESTP) nanomedicine to accumulate actively in calcified arteries. Zhang et al.¹⁶ engineered *Asparagus cochinchinensis*-derived EVs by inserting ligand-free DSPE-PEG into the lipid bilayer membrane's surface under the conditions of vortex and ultrasound. The altered EVs outperformed the unmodified EVs in terms of blood circulation time, tumor-targeting capability, and growth inhibition of HepG2 tumors. In addition to DSPE insertion, some targeted substances can be inserted directly into the lipid layer of PDEVs. Zhang et al.¹⁷⁵ engineered ginger-derived EVs through direct physical insertion. They modified folic acid into the lipid layer of ginger-derived EVs without introducing DSPE-PEG. Moreover, Zhuang et al.²⁰³ engineered grapefruit-derived EVs by physically mixing folic acid onto their surface. Modifying these EVs with folic acid can specifically target gliomas. By attaching itself to folic acid receptors on the surface of glioma cells, it strengthens the anti-tumor activities of the cells.

In addition to physical insertion, electrostatic adsorption is a common physical modification strategy for PDEVs²²⁹⁻²³¹. The negative surface charge of PDEVs provides an electrostatic adsorption site for cationic polymer^{229,230}. Tang et al.²¹² modified grapefruit-derived EVs by attaching targeting aptamers to

polyethyleneimine, which was created using the intrinsic electrostatic affinity between positive and negative charges.

4.3. Chemical modification

Chemical surface modification of EVs involves specific chemical reaction processes, which ensure strong specificity and efficiency. A chemical reaction can functionally modify the surface of a drug carrier by attaching the targeting ligand²³².

The PDEV membrane, which comprises a diverse range of lipids and proteins, offers numerous free amino and carboxyl groups²³³. Based on this characteristic, researchers have developed chemical modification techniques for PDEVs. Long et al.²⁰⁹ conjugated the amino groups of orange-derived EVs and carboxyl groups of heparin-RGD by an amidation reaction and constructed a targeted nanodrug system based on cRGD. Compared to unmodified EVs, the engineered EVs exhibited remarkable penetration ability in ovarian cancer and better anti-ovarian cancer effects. Similarly, Xiao et al.²⁰⁷ engineered lemon-derived EVs by chemical modification. In their study, heparin was modified on the surface of lemon-derived EVs through chemical crosslinking; the modified EVs showed excellent cell uptake capacities and overcame cancer's multidrug resistance. Guan et al.²¹⁴ engineered mulberry leaf-derived EVs by amide reaction. The cyclic-RGD was changed on the surface of mulberry leaf-derived EVs by chemical insertion, which prolonged the half-life of thrombolytic drugs and improved the thrombolytic effect at the thrombus. Xiao et al.¹⁸¹ and Niu et al.²⁰⁷ used chemical surface modification methods to surface grapefruit- and lemon-derived EVs, respectively, resulting in engineered EVs that allowed them to target ovarian cancer and glioma.

In addition to direct chemical surface modifications, innovative chemical modifications can be harnessed in biological orthogonal reactions to enhance the *in vivo* targeting of PDEVs. Incorporating hydrophobic dibenzocyclooctyne (DBCO) groups into the surface of PDEVs can create a powerful tool for precise targeting²³⁴. These modified PDEVs can be selectively targeted *in vivo* through a biological orthogonal reaction facilitated by the azide groups of DBCO. Furthermore, the cellular process of glucose metabolism induces the expression of azide groups on the membrane surface, enabling continuous bioorthogonal interaction with DBCO groups. This strategy allows for sequential drug delivery and maximizes the therapeutic impact of the target medication^{235,236}.

4.4. Membrane hybridization

Membrane fusion modification technology is derived from the biological hybrid nano delivery system, which refers to a delivery system obtained by fusing the traditional delivery system with the cell membrane or EVs²³⁷. The fusion nanoparticles can overcome the shortcomings of a single-drug delivery system²³⁷. Liu et al.²³⁸ first produced multifunctional hybrid membrane nanovesicles. In their study, red blood and platelet-derived cell membranes were fused into hybrid membranes under ultrasound conditions²³⁸. Membrane fusion PDEVs were first used to prepare phytochemically engineered bacterial outer membrane vesicles²¹⁶. Zhuang et al.²¹⁶ fused the thylakoid membrane derived from spinach leaves with the cell membrane derived from bacteria to prepare the bacteria-plant hybrid nanovesicles. This hybrid membrane technology may have a synergistic effect on the elimination of primary solid tumors. It stops the cancer from metastasizing. The

Table 5 PDEVs as delivery vehicles for cancer therapy.

PDEV	Anti-tumor drug	Loading method	Advantage	Ref.
Grapefruit-derived EVs	HSP70.	Sonication	Boost colonic carcinoma cell absorption.	201,202
	Doxorubicin, heparin nanoparticles.	Co-incubation	Enhance the uptake of glioma cancer cells.	181
	miRNA.	Sonication	Provide targeting capability.	203
	JSI-124, paclitaxel, siRNA.	Sonication	Improve security and provide targeting capability.	126
Ginger-derived EVs	10-Hydroxycamptothecin (HCPT).	Co-incubation	Sustain a consistent HCPT concentration <i>in vivo</i> and improve the delivery efficiency.	204
	Doxorubicin.	Sonication	High loading efficiency and pH-dependent release.	31,175
	siRNA.	Co-incubation	High production yield, high drug loading, low cost, and targeting capability.	116
Kiwi-derived EVs	Sorafenib.	Sonication	Enhance HepG2 cell absorption and reduce liver side effects.	205
	siRNA.	Co-incubation	Improve security and provide targeting capability.	200
Bitter melon-derived EVs	5-FU.	Sonication	Boost 5-FU's cytotoxicity and diminish drug resistance.	103
Aloe-derived EVs	Indocyanine green.	Co-incubation	Simplify the preparation, reduce production cost, protect the drug, and prevent drug leakage.	111
Tea leaf-derived EVs	Doxorubicin.	Sonication	Simplify the preparation and improve the delivery efficiency.	206
Lemon-derived EVs	Doxorubicin.	Co-incubation	Enhance the uptake of the Varian cancer cells	207
Acerola-derived EVs	miRNA.	Co-incubation	Low cost, simplifies preparation, and protects against RNase, strong acids, and bases.	208
Orange-derived EVs	Doxorubicin, heparin nanoparticles.	Co-incubation	Enhance the transcytosis capability of ovarian cancer cells.	209
Curcuma-derived EVs	Doxorubicin.	Co-incubation	Provide stable controlled drug release properties and targeting capability.	210
<i>Brucea javanica</i> -derived EVs	miRNA.	Co-incubation	Dual role in synergistic inhibition of angiogenesis and tumor development in therapy.	115
Broccoli-derived EVs	miRNA.	Co-incubation	Improves extracellular RNA stability and delivery.	146,194
Cabbage-derived EVs	Doxorubicin, DNA.	Co-incubation	Low cytotoxicity, multifunctionality, low production costs and high yields.	83
<i>Dendropanax morbifer</i> -derived EVs	Tyrosinase-related proteins (TRPs).	Freeze thawing	Small size, low toxicity, high absorption rate.	148
Tea flowers-derived EVs	Cells.	Co-incubation	Good biocompatibility and fewer side effects.	58

HSP70, Heat Shock Protein 70; JSI-124, cucurbitacin I; 5-FU, fluorouracil; RNase, ribonuclease.

preparation strategies for getting hybrid PDEVs can be summarized into three main categories: physical, chemical, and chimera (Fig. 5)²³⁹.

Co-extrusion is the most commonly used preparation method, which is simple to operate and can be achieved by extruding a press. When the cell membrane from two different cell sources or the nanoparticles containing the membrane structure are passively passed through the filter membrane of the specific pore size, the fusion nanovesicles with uniform particle size can be formed²¹¹. Huang et al.¹⁴³ prepared hybrid grapefruit-derived EVs using this method. Their study prepared multifunctional fusion nanovesicles

by co-extruding grapefruit-derived EVs and mesenchymal stem cell membranes loaded with immunosuppressant CX5461. The fused nanovesicles showed good biosafety and focal targeting and played a synergistic role in regulating the immune microenvironment. The membrane fusion technique can realize the biological orthogonal targeting of plant vesicles *in vivo*. Lu et al.²²¹ prepared hybrid exocarpium citri grandis-derived EVs, demonstrating exceptional targeting capability by bioorthogonal alteration. In addition to improving plant vesicles' *in vivo* targeting and safety, membrane fusion strategies can be used to engineer personalized cancer immunizations that prevent relapse and

Table 6 Engineering strategies for cancer therapy.

Engineering strategy	Source	Modification process	Purpose	Ref.
Physical modification	Grapefruit	(i) Induce targeted peptides <i>via</i> DSPE-(PEG)2000-maleimide.	(i) Target the site of vascular calcification.	32
		(ii) Induce targeted aptamers <i>via</i> DSPE-(PEG)2000-maleimide.	(ii) Target BBB endothelial cells.	211
		(iii) Induce targeted aptamers <i>via</i> polyethyleneimine.	(iii) Target breast cancer.	212
		(iv) Folic acid coating.	(iv) Target brain cancer cells.	203
	<i>Asparagus cochinchinensis</i> (Lour.) Merr.	(i) PEGylation <i>via</i> DSPE-PEG.	(i) Extend bloodstream duration and enhance tumor site accumulation.	16
	Corn	(i) PEGylation <i>via</i> DSPE-PEG.	(i) Extend blood circulation duration and enhance accumulation at tumor locations.	213
	Ginger	(i) Directly insert folic acid into the lipid layer.	(i) Target colon cancer cells.	175
		(ii) Link with fucoidan <i>via</i> the electrostatic of ϵ -PLL.	(ii) Target P-selectin.	31
	Curcuma	(i) Induce targeted antibody targeting death receptor 5 (DR5) <i>via</i> DSPE-PEG 2000-NH ₂ .	(i) Target senescent tumor cells.	210
	Orange	(i) Link with cRGD-targeted DOX nanoparticles <i>via</i> an amidation reaction.	(i) Target ovarian cancer cells.	222
Chemical modification	Lemon	(i) Link with heparin-cRGD <i>via</i> an amidation reaction.	(i) Boost DOX-resistant ovarian cancer's absorption efficiency.	207
	Mulberry leaf	(i) Link <i>via</i> an amidation reaction with cRGD.	(i) Target the site of the tumor and prolong the blood circulation time.	214
	Grapefruit	(i) Link with borneol <i>via</i> an amidation and esterification reaction.	(i) Improve drug absorption through the nasal mucosa.	215
		(ii) Link with pH-sensitive DNAs <i>via</i> an amidation reaction.	(ii) Target glioma cells and ensure highly efficient drug loading.	181

Gene modification	Kiwi fruit	(i) Induce siRNA, AF647, and EGFR aptamer.	(i) Target EGFR-mutant NSCLC and silence specific genes.	200
Membrane hybridization	Spinach	(i) Combine with outer membrane vesicles from <i>Escherichia coli</i> MG1655.	(i) Improve targeting accuracy and strengthen specific immune responses.	216
	Ginseng	(i) Integrate with the membrane derived from excised autologous tumors.	(i) Boost tumor cell immunogenicity and amplify targeted immune reactions.	217
		(ii) Fuse with the neutrophil membrane.	(ii) Targeting of lung tissues.	218
	Grapefruit	(i) Merge with CCR6 ⁺ nanovesicles from modified gingival MSC membranes.	(i) Improve targeting accuracy and immunomodulatory functions.	143
		(ii) Combine with active leukocyte membranes that are abundant in receptors linked to inflammation.	(ii) Target inflammatory tumor tissues.	219
	<i>Exocarpium Citri grandis</i> (E. C. grandis)	(i) Fuse with mesenchymal stem cell membrane-derived nanovesicles.	(i) Target the heart transplantation site.	221
	Lemon	(i) Fuse with 4T1 cancer cell membrane fragments.	(i) Target the homologous tumor.	220

DSPE-PEG, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-polyethylene glycol; BBB, blood–brain barrier; ϵ -PLL, ϵ -poly-L-lysine; DOX, doxorubicin; AF647, alexa fluor 647; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; CCR6, cc motif chemokine receptor 6.

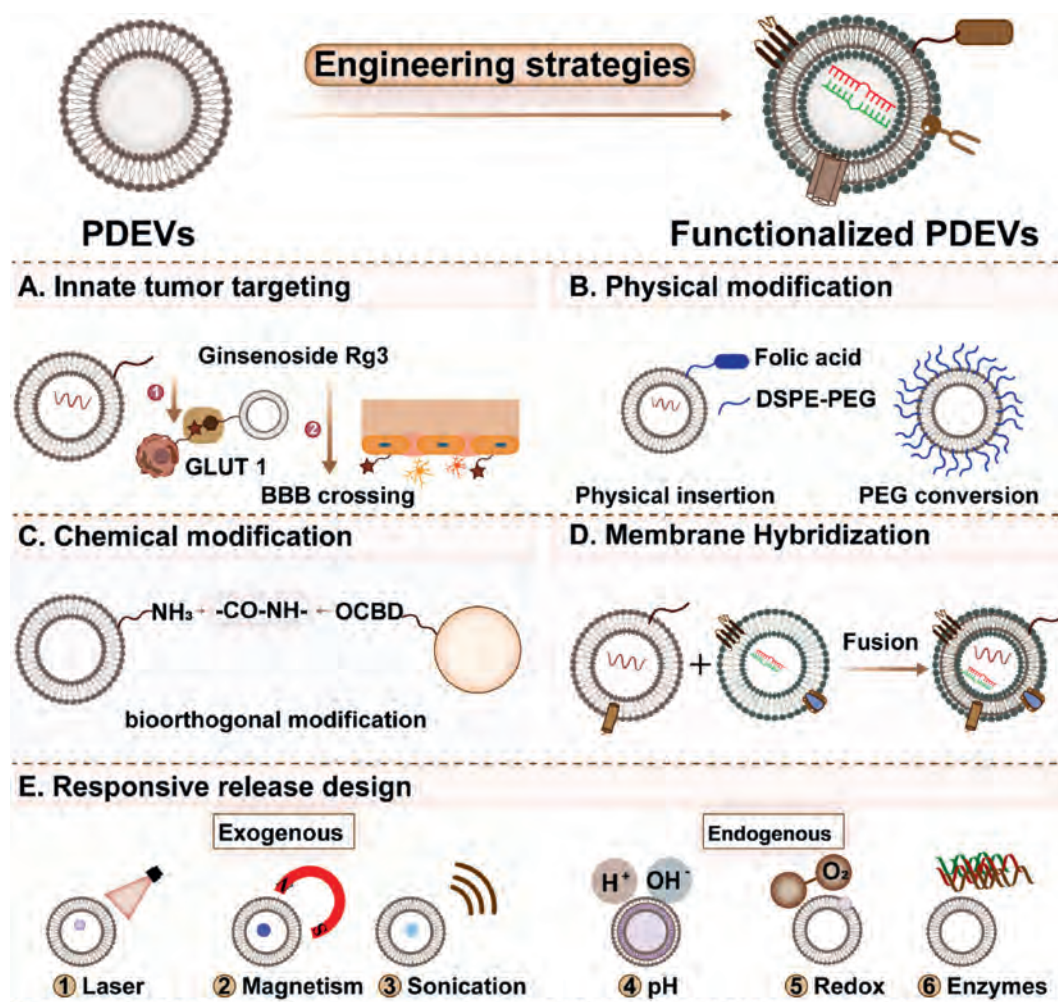


Figure 4 The engineering strategies of PDEVs.

spread²¹⁷⁻²¹⁹. Wang et al.²¹⁷ successfully developed a membrane fusion cancer vaccine by membrane fusion. The fusion vesicles were obtained from ginseng-derived EVs and the cell membrane of the resected autologous tumor using the co-extrusion method. This personalized tumor vaccine based on PDEVs can effectively reduce the possibility of postoperative tumor recurrence and metastasis and shows good biocompatibility.

Freeze-thaw methods can also be used to make fused nanovesicles. Different nanovesicles can break and recombine under different temperature cycling conditions, forming fused nanovesicles. Compared with the co-extrusion strategy, this method has obvious shortcomings. For example, various temperatures will affect the efficiency of membrane fusion and cannot guarantee the size of the particle. It is easy to obtain nanoparticles with uneven particle size. The ultrasonic fusion strategy refers to using ultrasound as an auxiliary method, whereby nanovesicles or cell membranes are subjected to ultrasound, resulting in rupture and reconstruction, ultimately leading to the formation of fused nanoparticles. Zhuang et al.²¹⁶ created an on-site tumor prophylactic *via* ultrasonic fusion of bacterial membrane vesicles and spinach thylakoid nanovesicles. Modified PDEVs exhibit enhanced tumor targeting. Compared with the first three physical preparation strategies, co-incubation is a mild membrane fusion

strategy. Two or more nanovesicles are mixed and incubated at 37 °C to form the fusion body^{211,240}.

In contrast to the physical preparation technique, the polyethylene glycol induction method offers the benefits of simplicity, high fusion efficiency, and the capacity to induce membrane fusion in a short period^{211,241}. However, due to the potential toxicity of polyethylene glycol, residual polyethylene glycol is particularly important in polyethylene glycol-induced membrane fusion. In addition to physical and chemical strategies, chimerism technology can achieve membrane fusion and obtain hybrids through biological chimerism^{211,242,243}.

Traditional single nano-vesicles often have single and limited functions, low drug loading capacity, limited targeting ability, limited ability to cross physiological barriers, and a relatively complex process of targeting and modification²⁴⁴. Membrane-fused nanometers can possess the advantages of two or more types of membranes^{237,244,245}. As delivery carriers, they have the following merits: (i) The preparation process is simple and can be mass-produced. Compared to the shortcomings of traditional EVs, such as low yield and significant limitations, fused nanovesicles can obtain sufficient yield to meet the requirements of experimental research through various preparation methods. (ii) Strong targeting. While the fused vesicles “inherit” the EVs of both

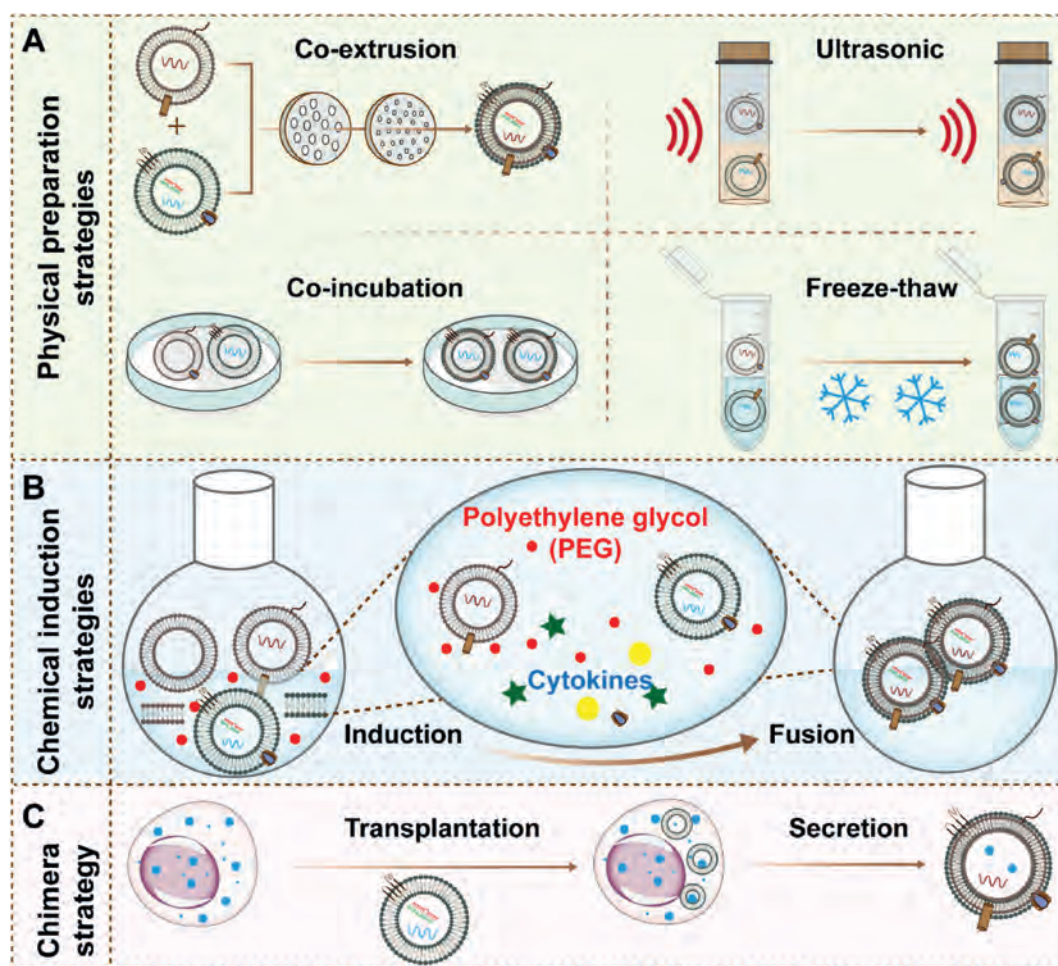


Figure 5 Three main strategies for membrane hybridization of PDEVs.

parent cells, they enhance the dual targeting effect. (iii) High biological safety. Fusion nanovesicles, which have clear preparation components and are loaded with biopharmaceutical components, reduce the safety risks associated with pure nanomedicines. The fused nanovesicles carrying chemical drugs have improved their bioavailability. (iv) Versatility. Fusion vesicles have overcome the natural EVs' single and limited functional limitations, broadening the application space of nano-delivery carriers and providing more therapeutic possibilities^{220,245-248}.

4.5. Responsive release design of PDEVs

Responsive design for cancer treatment refers to the responsive design of drug delivery systems and tumor treatment strategies based on the unique physicochemical properties of the endogenous tumor microenvironment. These properties include high levels of enzymes, glutathione, reactive oxygen species, or external stimulating factors. These features are beneficial to achieving more precise and effective tumor treatment. Currently, the commonly used response design types can be divided into endogenous response design and exogenous response design.

4.5.1. Endogenous response design of PDEVs

There are noticeable microenvironmental differences between tumors and normal tissues, including pH, ROS, GSH, and enzyme

profiles²⁴⁹. The high level of glycolysis and highly vascularized tumor cells lead to a tumor microenvironment with low pH, low oxygen, high ROS, high GSH, and overexpression of various enzymes, including proteases, lipases, matrix metalloproteinases, etc. Using these abnormal indicators in the tumor microenvironment, the delivery platforms of PDEVs for pH, ROS, GSH, enzymes, and hypoxia response pairs can be designed²⁴⁹. For instance, Lu et al.²²¹ prepared an innovative ROS response system by incorporating DBCO-NHS onto the surface of *Exocarpium Citri grandis*-derived EVs. In the ROS-enriched environment, ROS-N₃ will be hydrolyzed. The N₃-DBCO facilitates the targeted accumulation of *Exocarpium Citri grandis*-derived EVs at the target part through a "click" chemistry reaction. This research offers a valuable approach to developing multifunctional PDEVs.

4.5.2. Exogenous response design of PDEVs

Endogenous response design is a practical pathway to achieve spatial control. However, this strategy falls short of efficient targeting in disease microenvironments where factor levels do not change significantly. Additionally, it has limitations in time control, as the drug is released immediately upon stimulation of the corresponding signal, which stimulates the delivery system. In contrast, the exogenous response strategy effectively addresses these shortcomings, offering excellent spatiotemporal control²⁵⁰. Endogenous response strategies include light, heat, magnetism,

electricity, and ultrasound, among others. Incorporating photosensitizers, thermosensitizers, sonosensitizers, or other stimuli-responsive materials into PDEVs makes it possible to achieve targeted drug delivery^{224,225}. Moreover, light-responsive delivery platforms of PDEVs can precisely control drug release by adjusting parameters such as wavelength, power, and irradiation time^{226,227}. Magnetic fields can penetrate deep into tissues. Drugs may be given to deep lesions in an exact and non-invasive manner by regulating the strength and direction of the magnet. The magnetically sensitive delivery carrier of PDEVs may be employed for locally focused treatment of malignancies²⁵¹. Ultrasound response is determined by the sensitivity of acoustically sensitive materials to ultrasound. It releases medications *via* the cavitation and mechanical properties of ultrasound²⁵¹. The delivery system of PDEVs with ultrasound response can be used for drug treatment in the deep part of malignant tumors.

4.6. Gene modification

Genetic manipulation involves gene modification, cellular introduction of genetic material, and protein-based delivery methods to encapsulate therapeutic agents within or present them externally on vesicles. This host-cell-based technique intuitively releases tailored EVs from their biological source and separates²³⁶. Engineering modification of PDEVs refers to the use of gene engineering technology to purposefully transform, edit, or regulate genes related to vesicle formation, structure, and function in plant cells. Gene modification alters the biological characteristics of PDEVs, influencing their stability and the expression of related proteins²⁵². In recent years, many EV vaccines based on genetic engineering technologies have been under clinical evaluation and have gained attractive results⁸. By performing gene editing on progenitor cells to overexpress antigen proteins or peptides, these antigens or protein-peptides are secreted into EVs through hijacking cellular mechanisms, resulting in engineered EVs containing antigens and thereby triggering antigen-specific immune responses²⁵³⁻²⁵⁵. PDEVs and MDVs offer several advantages, such as good biocompatibility, low immunogenicity, natural RNA and protein carriers, unique immunomodulatory properties, and flexible modifiability, making them a focus of cancer vaccination⁸. For instance, Miyamoto et al.²⁵⁶ designed a peptide-based gene delivery system that effectively transfected callus cells by enhancing the endocytosis and cytoplasmic translocation of the vector peptide/DNA complex, thereby achieving gene transfection in the plant system.

Although gene modification shows promise in treating plant vesicle cancer, it remains in the experimental stage. Much pre-clinical and clinical research is still needed to achieve genetically engineered PDEVs for human health and cancer treatment.

Engineered PDEVs have a promising application prospect for cancer, and its advantages are mainly reflected in the following six aspects: (i) precise drug delivery, by delivering medications precisely to the lesion site, the modified PDEVs may prevent injury to healthy cells and improve the medications' safety and therapeutic effectiveness; (ii) improve medication use, PDEVs' double-layer membrane structure may shield the pharmaceuticals they contain from environmental disruptions, increasing the drugs' stability and bioavailability; (iii) the smaller particle size of PDEVs facilitates easier passage through the cell membrane and into the interior of the cell, which increases medication absorption and usage inside the cell; (iv) minimizing drug side effects: by precisely delivering medications to the lesion site instead of distributing them

throughout the body, tailored PDEVs may lessen the adverse effects of medications on healthy tissues; (v) when compared to the usage of pharmaceuticals alone, drug-adjuvant integration results in a greater amount of small molecule active compounds in designed PDEVs. Drugs may display multiple actions after loading, increasing their therapeutic worth and promising future; (vi) By constructing personalized vaccines, PDEVs can be membrane fused with autologous tumor cell membranes to obtain engineered personalized tumor vaccines and inhibit tumor recurrence. However, some challenges need to be addressed, such as limited characterization methods, the lack of standardized production standards, and the absence of low-cost engineering processes²⁵⁷.

The four PDEV-based engineering techniques may significantly expand the use of PDEVs in cancer by increasing the accumulation of medications at tumor sites, decreasing their toxicity and adverse effects, and improving their bioavailability. Physical alteration is a popular modification technique that offers the benefits of ease of use and minimal harm to the membrane structure when compared to chemical modification, membrane fusion, and genetic engineering²⁵⁸. However, at the moment, its primary use is internal vector change, which may impact the subsequent loading of medications^{251,259}. Chemical modification is more effective than physical modification, but the chemical reagents used in this process may affect the active components of the natural membrane²⁶⁰⁻²⁶². Chemical modification strategies endow PDEVs with active targeting or environmentally responsive release capabilities through the surface coupling of peptides, antibodies, or responsive molecules. By releasing medications in reaction to low pH, high ROS, and high GSH levels in the tumor microenvironment, this engineering approach has shown notable benefits in the local therapy of solid tumors^{260,263}. Although genetic engineering has many drawbacks, including technical complexity, high expense, and restricted application to pre-isolated exosomes, it can edit PDEVs more accurately than physical and chemical modifications. Furthermore, uncontrolled toxicity or immunogenicity may result from the gene transfection procedure^{30,264,265}. As a special engineering strategy, membrane fusion can achieve the fusion of PDEVs with drug-loaded liposomes, tumor cell membranes, and immune cell membranes in the tumor microenvironment, thereby achieving tumor targeting while further increasing drug loading. Membrane fusion is a promising nanoscale-targeted drug delivery technology that combines the benefits of liposomes and EVs as compared to physicochemical alteration and genetic engineering. Nevertheless, it has several drawbacks, including poor fusion efficiency and potential drug leakage during the fusion procedure²⁶⁶.

Even if many engineering techniques may successfully target tumors and enhance the therapeutic impact of cancer, more basic research, safety, and preclinical investigations are required to accomplish clinical transformation in the future.

5. Engineered PDEVs as nanocarriers for combinational cancer immunotherapy

Cancer immunotherapy stimulates the patient's defenses to initiate an anti-cancer response²⁶⁷. Compared with traditional radiotherapy and chemotherapy, immunotherapy has better safety and fewer side effects²⁶⁸. Chimeric antigen receptor T cell immunotherapy, immune checkpoint blocking treatment, and cancer vaccines are the three primary components of cancer immunotherapy.

Chemotherapy, light therapy, heat therapy, and gene therapy are additional anti-tumor therapies that can cause tumor cell death, generating damage-related molecular patterns and tumor-related antigens. These therapies can also cause tumor immunogenic death, improve T cell activation and DC maturation, and trigger an immune response against the tumor. Together with cancer immunotherapy, this anti-tumor immune response may strengthen the anti-tumor immune response and prevent tumor spread and recurrence²⁶⁹.

Cancer combination therapy is more effective than single therapy and can effectively overcome multidrug resistance in tumor cells^{270,271}. Research indicates that modified EVs can deliver two or more anti-tumor medications to the tumor site, resulting in a combination of anti-neoplastic impact²⁹. When used in conjunction with cancer immunotherapy, the modified PDEVs may have a synergistic anti-tumor effect due to their superior anti-tumor efficacy^{29,272}.

An overactive immune system may boost the immunological response, and immunotherapy targets a variety of targets. However, it may also harm systems and organs, including the liver and heart. Additionally, immune checkpoint blocking therapy can improve immune function while targeting normal cells, potentially impacting the body's overall health. The environment in which tumors grow is usually an immunosuppressive microenvironment with high levels of immune factors and immune cells, which can facilitate the immune escape of tumor cells. High vascularization, low pH, and a hypoxic microenvironment hinder immune cell invasion and tumor-killing²⁷³. The complex immunosuppressive microenvironment and low levels of immune cells significantly limit the effectiveness of immunotherapy. Hence, developing integrated nano-delivery systems is crucial for boosting immunotherapy through combinatorial treatments. PDEVs exhibit good biocompatibility, a nano-size, and practical tissue penetration ability. They can be engineered to composite particles to achieve immunotherapy and other therapies to inhibit tumor growth (Fig. 6). Engineered PDEV-mediated

immunotherapy synergistically leverages distinct treatment benefits, mitigating individual therapeutic drawbacks (Table 7) 111,115,160,206,210,217,274-279.

5.1. Immunotherapy and chemotherapy synergistic therapy

According to recent studies in tumor immunology, conventional chemotherapy may also elicit immune responses against the tumor and provide long-lasting tumor suppression²⁸⁰⁻²⁸². Doxorubicin, oxaliplatin (OXA), and paclitaxel are examples of conventional chemotherapy medications that may induce immunogenic cell death (ICD), an aberrant cell death that triggers the adaptive immune system to provide an efficient anti-tumor response²⁸³⁻²⁸⁶. The generation of damage-related molecular patterns during the ICD process of tumor cells releases ATP, calreticulin (CRT), and high mobility histone B1 (HMGB1), activates effector T cells, natural killer cells, or antigen-presenting cells, and sets off both innate and adaptive immune responses²⁸⁷. The immunosuppressive microenvironment may be reversed by some PDEVs, such as ginseng, *Artemisia annua*, and *Platycodon grandiflorum*-derived EVs, which may enhance NK cell activity. This activity then regulates immune cells in the tumor microenvironment, encouraging the polarization of anti-tumor macrophages and upregulating macrophage levels^{142,160,288}. It is thus possible to achieve a combination of chemotherapy medications and PDEVs to enhance the anti-tumor immune response by selecting PDEVs with immunomodulatory properties to deliver chemotherapy drugs.

The limitations of conventional chemotherapy may be addressed by combining immunotherapy as a potential treatment approach, although immunotherapy is not always successful in stimulating the immune response^{38,289}. Additionally, combining the two treatments might lower the amount of chemotherapy medications, which lessens the harmful side effects of the medications^{38,280}. The primary function of altered PDEVs in accomplishing the combination of immunotherapy and chemotherapy will be briefly covered in this section. At present, the realization of

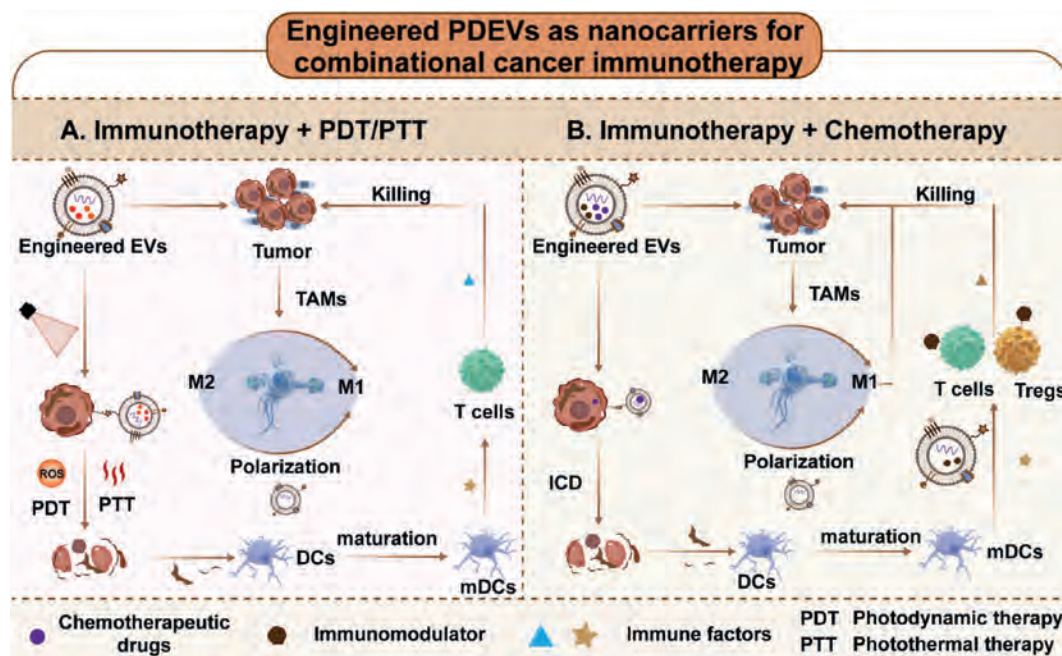


Figure 6 The process of engineered PDEVs as nanocarriers for combinational cancer immunotherapy.

Table 7 The application of engineered PDEVs as nanocarriers for combinational cancer immunotherapy.

Combination therapy	Source	Application	Ref.
Photodynamic therapy-immunotherapy	<i>Hypericum perforatum</i>	(i) Serve as a natural photosensitizer to induce apoptosis in tumor cells; (ii) Use the inherent fluorescence to monitor real-time treatment efficacy.	274
Photothermal therapy-immunotherapy	Aloe	(i) Reduce the toxicity of photothermal agents; (ii) Achieve transdermal delivery for skin cancer treatment.	111
	Aloe	(i) Deliver photothermal agents; (ii) Regulate the immune microenvironment.	275
	Ginger	(i) Conjugate with electrodynamic Pd–Pt nanosheets; (ii) Combine photothermal and electrodynamic therapy.	276
Chemodynamic therapy-immunotherapy	Tea-leaf	(i) Provide binding sites for metal ions; (ii) Target tumor-associated macrophages; (iii) Combine chemo-dynamic therapy and immunotherapy.	206
Metabolism regulation-immunotherapy	<i>Citrus limon</i>	(i) Downregulate acetyl-CoA carboxylase processes; (ii) Mediate lipid metabolism in cancer cells.	277
	<i>Astragalus</i>	(i) Promote anaerobic metabolism in the tumor microenvironment.	278
Chemotherapy-immunotherapy	Citrus fruit	(i) Contain anti-tumor bioactive components; (ii) Load chemotherapy drugs and immune adjuvants; (iii) Combine chemotherapy and immunotherapy.	279
	Ginseng	(i) Co-deliver autologous tumor antigens and immune adjuvants; (ii) Contribute to immune regulation.	217
	Curcuma	(i) Co-deliver chemotherapy drugs and antibodies; (ii) Combine chemotherapy and immunotherapy.	210
Gene therapy-immunotherapy	<i>Brucea javanica</i>	(i) Deliver autologous miRNA for cancer therapy.	115
	<i>Artemisia annua</i>	(i) Deliver autologous mitochondrial DNA for cancer therapy.	160

Acetyl-CoA, acetoacetyl coenzyme A.

chemotherapy combined with immunotherapy through engineered vesicles mainly includes the following three aspects: (i) as a delivery carrier, it can be used to co-deliver chemotherapy drugs, immune adjuvant or immune checkpoint blockers to the tumor location to suppress tumor progress collaboratively; (ii) some PDEVs with immune regulation ability, such as ginseng-derived EVs, can build personalized tumor vaccines with tumor cell membranes and carry chemotherapy drugs to achieve combined therapy; (iii) when utilized in conjunction with chemotherapeutic medications, PDEVs with anti-tumor action may function as delivery vehicles for these medications^{217,279,290}. For example, Li et al.²⁷⁹ prepared engineered citrus fruit-derived EVs as a delivery carrier of doxorubicin to achieve chemotherapy combined with immunotherapy, effectively inhibiting the growth and recurrence of glioma (Fig. 7A and B). Citrus fruit-derived EVs that contain heparin sodium and chimeric ANG peptide may transport doxorubicin to the tumor location when heparin sodium's focused activity is in effect. Hesperidin, vitamin C, vitamin E, and the immune adjuvant ANG all work together in EVs to support the anti-tumor immune response. Doxorubicin-induced immunogenic death of the tumor can further stimulate the immune response. Combined chemo-immunotherapy exhibited superior tumor growth inhibition versus monotherapy.

Wang et al.²¹⁷ constructed personalized cancer vaccines using EVs-derived from ginseng, and membrane-fused PDEVs were utilized for the co-delivery of autologous tumor antigens and immune adjuvants (Fig. 7C). The engineered ginseng EVs showed excellent delivery performance. They enhanced the phagocytosis function of dendritic cells, activated toll-like receptor 4 to promote the growth of DCs, and then activated T cells. The engineered personalized vaccine can effectively inhibit tumor recurrence and metastasis. Guo et al.²¹⁰ used DSPE-PEG2000 as an intermediate to modify turmeric-derived EVs and modified the target death receptor 5 onto the surface of these EVs. The modified EVs were loaded with doxorubicin through co-incubation. The engineered delivery system can target glioma cells under the action of targeted receptors, induce tumor cell death, activate natural killer cell activity by down-regulating senescence-related factors, and synergistically inhibit glioma cell growth.

5.2. Immunotherapy and photothermal therapy synergistic therapy

Immune off-target and low immune response are the main challenges of immunotherapy alone. To generate tumor antigens and immune-related chemicals, photothermal treatment can directly

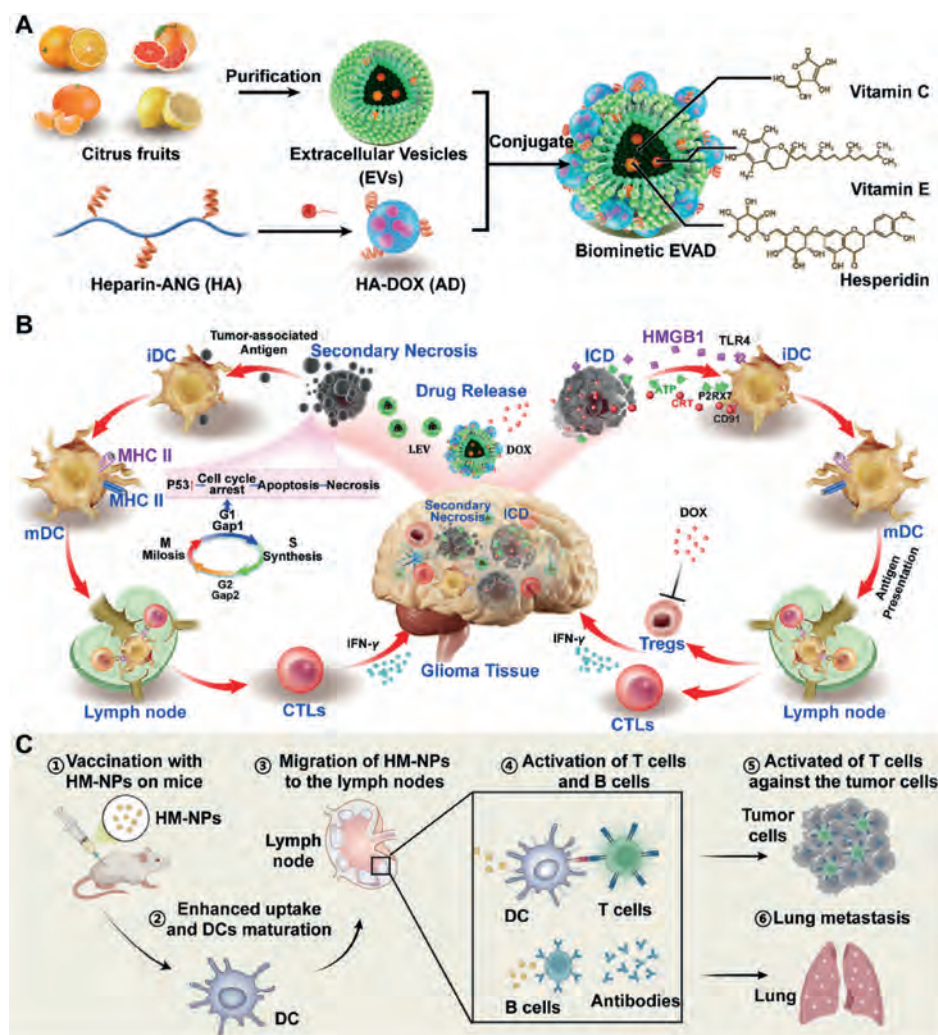


Figure 7 The application of PDEVs in realizing immunotherapy combined with photothermal therapy. (A) Assembly diagram of pH-sensitive lemon-based nanoparticles for treating glioma. (B) Reversal of immunosuppressive microenvironment mechanism by secondary necrosis and immunogenic death induced by EV and DOX. Reprinted with permission from Ref. 279. Copyright © 2024, Elsevier. (C) The immunoenhancement of HM-NPs. Reprinted with permission from Ref. 217. Copyright © 2024, Wiley.

destroy tumor cells. It can also encourage DC cell maturation and T cell activation, thereby enhancing anti-tumor immunity^{280,291}. The immune response induced by photothermal therapy is easily hindered by stromal cells and cannot completely kill tumor cells, which are prone to relapse. Immunotherapy combined with photothermal therapy can compensate for the recurrence of photothermal therapy alone, and the two can exhibit a synergistic anti-tumor effect, which has a broad application prospect. Accurate targeting of tumor sites is essential to ensure photothermal combined immunotherapy. The use of PDEVs with immunomodulatory capabilities loaded with photosensitizers to target the tumor site is known as photothermal treatment in conjunction with immunotherapy based on tailored PDEVs^{29,292,293}. Under the irradiation of a laser, local temperature rise induces tumor death and releases damage-related molecular patterns, which increase antigen-presenting cells' production of MHC-I, MHC-II, and costimulatory molecules (CD80 and CD86) to improve antigen cross-presentation and T cell activation and further accomplish the joint destruction of tumor cells. Zeng et al.¹¹¹ isolated nanovesicles from *Aloe vera* as delivery carriers of the photothermal

agent indocyanine green and performed engineering treatment by the co-incubation method to obtain drug-carrying nanovesicles decomposed in response to temperature. After drug administration, under laser irradiation, the temperature of the nanoparticles carrying the drug rises, causing cracks, which promotes the heat conversion of the photothermal agent and results in the immunogenic death of melanoma. In order to achieve the synergistic destruction of tumor cells by photothermal and immunotherapy, immunogenic death may produce tumor antigens to stimulate anti-tumor immune responses and create injury-related molecular patterns. Therefore, engineered plant vesicles with immune adjuvants, tumor antigens, and loaded photothermal agents can combine immunotherapy and photothermal therapy to inhibit tumor growth and recurrence.

5.3. Immunotherapy and photodynamic therapy synergistic therapy

Antigen presentation, DC cell maturation, T cell activation, and tumor-killing are the key processes of the anti-tumor immune

response. Each stage is critical to the immune response²⁸⁰. Immunotherapy can be challenging to implement throughout the immune response. By improving antigen presentation, encouraging cytokine release, and upregulating HSP, photodynamic treatment may strengthen the immune response^{29,269,291}. Therefore, combining immunotherapy and photodynamic therapy can synergistically activate the immune response in the body and synergistically inhibit the growth and recurrence of tumor cells²⁹⁴. Photodynamic combined immunotherapy based on PDEVs refers to the use of PDEVs, which have the characteristics of natural photosensitizers, as a delivery carrier to deliver substances such as immune adjuvants and immune checkpoint inhibitors. Under the irradiation of specific wavelengths, ROS are generated to induce tumor cell death and activate anti-tumor immune responses, thereby achieving synergistic tumor killing. Ma et al.²⁷⁴ discovered that hypericum-derived EVs with photosensitive properties can produce many reactive oxygen species under laser irradiation, inhibiting melanoma growth. Reactive oxygen species may trigger the anti-tumor immune response and induce apoptosis. As a result, it is possible to design and use plant vesicles with photosensitive activity as immunotherapy carriers, successfully combining immunotherapy with photothermal treatment.

5.4. Immunotherapy and chemo-dynamic therapy synergistic therapy

Chemo-dynamic therapy is a new method of treating cancer that creates deadly hydroxyl radicals by using Fenton/Fenton-like processes. Through the interaction of metal ions (Fe^{2+} , Cu^{+} , Mn^{2+} , and Mo^{4+}) with the overexpressed H_2O_2 present in tumors, this mechanism targets and kills tumor cells preferentially^{295,296}. Nevertheless, the Fenton reaction's efficiency is higher than that of CDT. The therapeutic effect of CDT may be severely hampered by elements like low H_2O_2 levels and the tumor microenvironment's slightly acidic pH²⁹⁷. Chemo-dynamic combined immunotherapy based on PDEVs involves modifying metal ions on the surface of PDEVs with immunoregulatory capabilities and conducting targeted modifications to induce Fenton/Fenton-like reactions at the tumor site while regulating immune cells, thereby achieving a combination of immunotherapy and chemo-dynamic therapy. For example, tea leaves-derived EVs include polyphenols on their surface that may be associated with Fe^{3+} . Engineered extracellular vesicles containing intrinsic galactose may cause targeted polarization of tumor-associated macrophages, primarily affecting macrophages within the tumor microenvironment²⁰⁶. By triggering antigen presentation and causing immunogenic cell death, doxorubicin may destroy tumor cells and elicit an immune response against the tumor.

Additionally, Fe^{3+} triggers the Fenton reaction, resulting in immunogenic cell death²⁰⁶. This multifunctional delivery system can present strong anti-tumor effects. The presence of active ingredients in PDEVs provides binding sites for metal ions, which helps avoid the need for chemical ligands and simplifies the synthesis process. This dramatically increases the possibility of using immunotherapy and chemo-dynamic therapy in tandem to treat cancer.

5.5. Immunotherapy and metabolism therapy synergistic therapy

Regulating the tumor immunosuppressive microenvironment is a promising therapeutic direction for improving

immunotherapy^{298,299}. The tumor immunosuppressive microenvironment contains complex components such as cells, factors, metabolites, blood vessels, and fibers. Metabolites in the microenvironment include tumor cells and immune cells^{280,300}. The high level of lactate metabolites in the microenvironment causes the immune microenvironment to have a low pH state. Lactic acid is the product of the glycolysis of tumor cells. Regulating the metabolism of tumor cells or immune cells can affect the growth of tumor cells. Metabolic reprogramming targeting tumors provides new therapeutic directions for cancer therapy^{301,302}. Many compounds from traditional Chinese medicine have shown potential in reversing the development of tumor cells and inducing their apoptosis by regulating metabolism³⁰³. For instance, acetyl-CoA carboxylase, a crucial enzyme in the glycolysis of tumor cells, is down-regulated by *citrus* and lemon-derived EVs, which prevents colorectal cancer cells from growing. Furthermore, research has shown that astragalus may suppress the growth of tumor cells and enhance cell metabolism in the tumor microenvironment^{278,304}. Therefore, selecting PDEVs with tumor metabolic regulation as the delivery vector of immunotherapy can realize the combination of immunotherapy and metabolic therapy.

5.6. Immunotherapy and gene therapy synergistic therapy

Cancer gene therapy refers to treatment methods that regulate the expression or replacement of cancer genes^{305,306}. Currently, the commonly used gene intervention methods primarily employ small interfering RNA or plasmids to suppress the activity of associated genes in cancer cells^{306,307}. To achieve combined immunotherapy and gene therapy, PDEV-based gene therapy and immunotherapy involve the selection of PDEVs with immunomodulatory capabilities to deliver small interfering RNAs and targeted modification, thereby intervening in the epigenetic heritage of tumor cells while regulating immune cells. PDEVs, a good drug delivery vector, may deliver small interfering RNA and plasmids. For example, *Brucea javanica*-derived EVs can effectively provide therapeutic miRNAs to 4T1 cells, promote ROS/Caspase-mediated apoptosis by regulating the PI3K/Akt/mTOR signaling pathway, and inhibit angiogenesis in the tumor microenvironment. This markedly hindered 4T1 cell proliferation and spread¹¹⁵. Furthermore, Liu et al.¹⁶⁰ found artemisinin-derived EVs could influence the phenotype of tumor-associated macrophages. They found the cGAS–STING pathway of macrophage was triggered by the internalization of artemisinin-derived EVs, and it encouraged tumor-associated macrophages to develop anti-tumor characteristics¹⁶⁰. Therefore, engineered PDEVs can serve as a co-delivery vector for immunotherapy in combination with gene therapy.

5.7. Cancer vaccines based on PDEVs

Immunotherapy harnesses the body's defenses to target aberrant or unhealthy cells, safeguarding normal tissue. When paired with other treatments, PDEVs, an outstanding delivery mechanism, may achieve the anti-tumor impact of immunotherapy. A novel avenue for enhancing the therapeutic efficacy of immunotherapy is provided by PDEVs, a carrier material with high biocompatibility that can be designed to accomplish tumor site targeting and responsive release in combination with treatment. Furthermore, PDEVs may be used to create gel delivery systems that transport antigens, encouraging T cell activation and DC cell maturation. For example, Yang et al.³⁰⁸ utilized ginseng and spinach-derived

EVs to construct functionality hydrogels, antigen peptide OVA was modified to the surface of spinach EVs through maleimide-mediated Michael addition reaction, and oxidized sodium alginate was further modified to ginseng EVs by Schiff base reaction³⁰⁸. After administration, the two engineered PDEVs are mixed and cross-linked to form a hydrogel in tumor tissue under Ca^{2+} chelation. The hydrogel can produce oxygen in the tumor microenvironment with a high level of hydrogen peroxide, reverse hypoxia-mediated immunosuppression, and promote the maturation of dendritic cells and activation of T cells together with OVA peptides, triggering a strong anti-tumor immune response to inhibit the growth of the tumor.

6. Pharmacokinetic study of PDEVs

Studying the safety and therapeutic use of PDEVs requires first understanding their pharmacokinetics *in vivo*. Pharmacokinetic studies of PDEVs are still in the early stages of basic research. In the current basic research, the pharmacokinetic analysis of PDEVs is still in the initial stage. Current studies have focused on metabolomics to analyze the primary components of PDEVs and their distribution *in vivo*, as well as their potential medicinal mechanisms. The body's metabolism and excretion process of PDEVs is not fully understood³⁰⁹. Based on the present study, we provide a rough overview of the metabolic processes in plant vesicles.

6.1. Absorption of PDEVs

Absorption is the initial process by which vesicles enter the body. Effective absorption is crucial for ensuring PDEVs enter the systemic circulation. The absorption mode of PDEVs is intricately linked to the method of administration^{42,310}. PDEVs are absorbed by the gastrointestinal tract mainly through oral administration^{52,113}. The primary absorption mechanisms of PDEVs can be categorized into five pathways: lattice protein-dependent endocytosis, niche protein-mediated uptake, giant cytosolic uptake, phagocytosis, and lipid raft-mediated internalization^{311,312}. In contrast to conventional small-molecule drugs that enter cells through passive diffusion, PDEVs and nanomedicines primarily enter the cells through endocytosis, allowing for effective accumulation in target cells³¹³. Cui et al.³¹⁴ discovered that lemon-derived EVs were taken up by endocytosis in 4T1 and HCC-1806 cells. In their *in vitro* experiments, the absorption mechanism of lemon-derived EVs was investigated by incubating tumor cells with lemon-derived EVs under different conditions and observing the colocalization result.

In addition to oral and intravenous administration, PDEVs can also be absorbed through transdermal and nasal routes. Due to its particular lipid bilayer structure, it can be absorbed by skin tissues and enter the dermis through free diffusion. Some recent reports indicated that broccoli, *Aloe vera*, and ginseng-derived EVs could promote skin regeneration by transdermal absorption³¹⁵⁻³¹⁷. Nasal absorption is a valuable technique for boosting the efficacy of PDEVs in treating brain diseases. Nasal delivery allows PDEVs to access the brain directly, bypassing the blood-brain barrier. Zhuang et al.²⁰³ used grapefruit-derived EVs to suppress the growth of brain malignancies *via* nasal delivery. The absorption efficiency of grapefruit-derived EVs was measured by fluorescent labeling; the result showed that these EVs could quickly reach the olfactory bulb, hippocampus, thalamus, and cerebellum.

Selecting the appropriate route of administration is essential to achieving efficient absorption of PDEVs. Recent research utilized tea leaves-derived EVs to treat breast cancer by administration and intravenous injection¹⁴⁵. Results indicated that oral administration of tea leaves-derived EVs did not result in significant adverse effects, unlike intravenous infusion, which induced liver and kidney toxicity. This suggests that oral absorption is more suitable for PDEVs.

The administration method affects the *in vivo* absorption of PDEVs³¹⁸. Currently, the most commonly used administration methods for PDEVs include oral administration and intravenous injection³¹⁸. Recent studies have shown that when administered orally, PDEVs are mainly concentrated in the gastrointestinal tract and can maintain good particle size and potential stability and anti-gastrointestinal digestion ability in the gastrointestinal tract¹⁸. Oral PDEVs are highly suitable for gastrointestinal diseases such as cancer and colitis^{318,319}. Therefore, oral administration is a key route of PDEV administration^{137,318}. However, the oral administration route often has the first-pass effect on the liver, which can be mitigated by selecting appropriate administration time and increasing the initial administration dose.

The intravenous injection administration method can effectively avoid the first-pass effect of the liver³¹⁸. PDEVs can directly enter the bloodstream to achieve tissue distribution and targeting outside the gastrointestinal tract. For example, after intravenous injection of *Panax notoginseng*-derived EVs, it can effectively achieve brain targeting³²⁰. After intravenous treatment, bitter melon EV may concentrate at the tumor site, have a reasonably extended half-life *in vivo*, and efficiently accumulate in the liver and spleen³²¹. However, it should be emphasized that intravenous administration is likely to produce liver and renal damage, as well as immunological activation^{40,145,317}. Therefore, based on the advantages and disadvantages of oral and intravenous injection, the specific and appropriate administration method can be selected according to different disease sites. At the same time, other administration methods, such as nasal administration, can also be chosen. The absorption mechanisms of PDEVs from various sources *in vivo* are complex and require more basic research and preclinical studies.

6.2. Distribution of PDEVs

The study of *in vivo* biological distribution is crucial for evaluating the safety and targeting of PDEVs. The distribution concentration of PDEVs in different organs will directly affect the efficacy of the drug. Factors such as surface composition, targeting modification, and method of administration influence whether PDEVs can effectively accumulate at the target site and reach therapeutic concentration. Studies have shown that ginger-derived EVs have intestinal targeting characteristics and may accumulate in the intestinal system following oral treatment. Teng et al.¹⁸ compared ginger-derived and grapefruit-derived EVs and found that lipid composition influenced the *in vivo* targeted distribution of PDEVs. Ginger-derived EVs, rich in PA, are preferentially absorbed by the lactic acid bacteria family, resulting in accumulation and staying in the intestine for a long time. Grapefruit-derived EVs, rich in PC, are preferentially absorbed by rumen bacteria, accumulating less in the intestine and more in the liver¹⁸. In addition, Gao et al.¹⁵² discovered that the galactose groups on the surface of *Morus nigra* L. leaves-derived EVs could specifically recognize the asialoglycoprotein receptor of liver tumor cells and achieve targeted distribution of the diseased liver through

“ligand–receptor” interaction. Garlic-derived EVs promote the distribution of CD98 receptors on the surface of hepatocytes through lectin II binding⁴⁹.

Meanwhile, different administration methods result in varying drug distributions. Oral medications are primarily distributed throughout the gastrointestinal system, while intravenous and intraperitoneal injections are typically administered to organs with high blood flow, such as the liver and spleen³²². For example, Cao et al.⁴⁰ compared the effects of different modes of administration of biodistribution in ginseng-derived EVs and found that intraperitoneal injection was mainly distributed in the liver, spleen, and gastrointestinal tract, intragastric administration was primarily in the gastrointestinal area, intravenous injection mostly distributed in the liver and spleen, and subcutaneous injection had lower levels in all organs. The intraperitoneal injection can achieve the targeted distribution of immune organs. Ou et al.⁹⁴ studied the distribution of *Catharanthus roseus* (L.) leaves-derived EVs in immunological organs such as the liver and thymus following intraperitoneal injection compared to oral and tail vein injections. Factors such as structural composition, modification strategy, and route of administration will affect the *in vivo* distribution of PDEVs. To improve the drug's bioavailability, the appropriate PDEVs and mode of administration should be selected according to the target organs and cells.

6.3. Metabolism of PDEVs

The metabolic investigation of PDEVs is still in the early stages of the current main research. Substance metabolism is called biotransformation and usually involves converting hydrophobic materials into polar solvents for excretion by the kidneys^{323,324}. The metabolic process of PDEVs primarily occurs in the liver and involves oxidation reactions^{325,326}. Researchers usually use fluorescent labeling to track the metabolic process of PDEVs in the liver and analyze the metabolic rate of PDEVs by detecting the fluorescence intensity³²⁷. Dysbiosis of the microbiota and disruption of the intestinal barrier can easily lead to intestinal diseases, which can affect the liver^{328–330}. PDEVs are commonly used to treat liver and intestinal diseases, and the gut–liver axis is a possible metabolic pathway. Oral PDEVs are transported through the portal vein to the liver, which secretes bile acids into the intestine. Metabolites from the intestine pass multiple layers of the intestinal barrier and reach the liver *via* the gut–liver axis. This interaction prolongs the metabolic time³³¹. The gut microbiota influences plasma levels of metabolites, including fatty acids, amino acids, and proteins. Grapefruit EVs were absorbed by DX5⁺ NK cells (10.9%) and F4/80⁺ cells (12.5%) in the spleen, as well as F4/80⁺ cells (4.65%), DX5⁺ NK (1.75%), and CD19⁺ B cells (1.63%) in the liver. The fluorescence signals in the liver and spleen remain strong on the twentieth day, indicating that they can be used for the long-term treatment of chronic diseases. Additionally, the placental barrier in pregnant mice can block the penetration of vesicles¹²⁶. Plant miRNAs were found in animal blood and tissues, and it was expected that miRNA from rice (miR168a) might target low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) in mouse liver, bind to LDLRAP1 mRNA, inhibit LDLRAP1, and impact lipid metabolism. Subsequent experiments have shown that plant miRNAs can regulate target gene expression in animals. In addition, it was found that this miRNA is absorbed and released into the bloodstream through the gastrointestinal tract. Its effect is stronger when encapsulated by vesicles,

which prompts the relevant metabolic processes of PDEVs that already carry miRNA³³².

6.4. Excretion of PDEVs

PDEVs excretion is the process by which PDEVs and their metabolites are eliminated from the body. The study on the excretion mechanism of PDEVs is still in progress, and the excretion study of PDEVs can reflect its effectiveness and potentially toxic side effects. Although the exact excretion pathway is unclear, PDEVs may be excreted through renal filtration, similar to EVs and exosomes in other organisms³³³. PDEVs are also eliminated directly by the liver. Liu et al.¹⁶⁰ investigated the distribution of EVs produced from *Artemisia annua* using fluorescence tagging. The findings demonstrated that in addition to being dispersed in the liver and target tumor tissue, a percentage of the *Artemisia annua* generated EVs are also distributed in the kidneys, and the fluorescence intensity decreased with time, indicating that a part of them is eliminated in the kidneys. In another study, the distribution of *Rhizoma Drynariae*-derived EVs was found in the liver, spleen, and femur, with almost no distribution in the kidney after intravenous injection and intraperitoneal administration³¹³.

Over the past decade, research on PDEVs has primarily focused on disease applications and the development of delivery vectors. Research on the absorption, distribution, metabolism, and excretion of PDEVs *in vivo* is limited, and associated mechanistic studies are also inadequate. The majority of studies on PDEVs focus on their absorption and distribution, with metabolism and excretion receiving less attention. Further research is needed to elucidate the metabolism and excretion of PDEVs *in vivo*, as well as the associated processes.

7. Clinical transformation of PDEVs

Unlike chemical drugs, PDEVs are derived from renewable plant sources, and their extraction process primarily utilizes physical methods. This results in waste mainly from biodegradable plant residues, leading to a lower environmental impact⁴². Therefore, plant vesicles are expected to offer more environmentally friendly options for drug production. Herein, we will discuss the development and critical issues associated with the scale-up production and clinical research of PDEVs (Fig. 8).

7.1. Challenges of scale-up the production of PDEVs

The large-scale production of PDEVs faces significant challenges, primarily due to the lack of relevant regulatory policies and industry standards¹³³. The variability in plant species, growth stages, and geographical regions necessitates a systematic evaluation of the stability of the original plant. This is especially crucial when PDEVs are utilized as therapeutic agents or drug carriers, as it is necessary to ensure that the products meet fundamental requirements for safety, efficacy, stability, and controllability³¹¹. Another major challenge in the scale-up production of PDEVs is the need for cost-effective separation methods. However, these methods can be expensive and may damage the vesicle structure, limiting their industrial viability⁵. In contrast, tangential flow filtration, which utilizes membrane separation, offers a solution to the challenges of low yield and lengthy processing times associated with ultracentrifugation³³⁴.

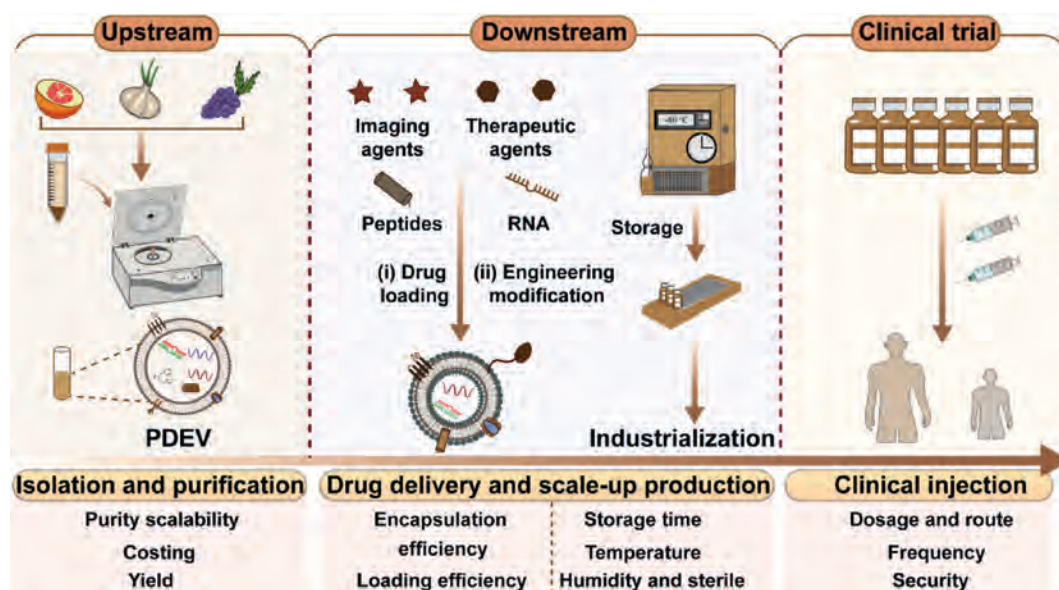


Figure 8 The development and critical issues of PDEVs-based nano-DDSs can be subdivided into upstream and downstream processing and preclinical study.

Additionally, scale-up production must ensure that each batch of PDEVs maintains uniformity and stability in terms of size, purity, and biological activity. Therefore, it is crucial to establish technical specifications that cover various aspects such as vesicle source, preparation, encapsulation, storage, and strict quality standards^{47,133}. The technology and processes for large-scale development represent a core focus in the research and application of PDEVs. Achieving large-scale production and application requires establishing a stable and efficient production process, as well as a robust quality control system.

7.2. Clinical research of PDEVs

In recent years, researchers have conducted many clinical trials on EVs. However, most of these trials have focused on MDVs, with relatively few clinical studies on PDEVs^{42,131,335,336}. Clinical studies on PDEVs have mostly focused on their use in medication administration and illness treatment over the last ten years. The paper summarizes the related clinical trials available on ClinicalTrials.gov (<https://clinicaltrials.gov/>), which include four clinical trials, as shown in (Table 8). In a Phase I trial (NCT01294072), grapes-derived EVs were used to deliver curcumin and evaluate the delivery efficiency of grapes-derived EVs in normal and colon cancer patients. Another preliminary clinical trial, NCT04879810, utilized ginger-derived EVs to deliver curcumin to treat Irritable Bowel Disease (IBD). Additionally, this study assessed the safety and acceptability of PDEVs in IBD

patients as well as the impact of ginger-derived EVs, either alone or in conjunction with curcumin, on symptoms in patients with refractory IBD. These two clinical studies validated the clinical safety of PDEVs as a carrier and showed the potential clinical use of PDEVs as a drug delivery vehicle. Furthermore, two additional clinical studies, NCT03493984 and NCT01668849, evaluated the potential of EVs produced from grapes to treat head and neck cancer and oral cataracts, respectively. Additionally, the potential of EVs derived from ginger and *Aloe vera* to decrease chronic inflammation in patients with polycystic ovarian syndrome. Although clinical trials and applications of PDEVs are still in their early stages, Current studies suggest they may have a significant impact on disease therapy and medication delivery.

Even with the exciting introduction of PDEVs in clinical trials, we're mindful of the challenges ahead for their widespread application. First, further exploration is needed regarding component analysis, extraction, separation methods, and the critical markers of PDEVs. Second, more investigation is needed to examine the pharmacokinetic behavior and *in vivo* safety of PDEVs. Third, further exploring how PDEVs treat diseases is essential for advancing medical research and improving patient care. Fourth, the potential interaction between drug molecules and PDEVs when used as a drug delivery system is unclear. Fifth, to better understand the *in vivo* mechanisms of PDEVs, a multidisciplinary approach that incorporates both macro and micro methods is required.

Table 8 The clinical trials of PDEVs.

PDEV	Application	Cargo	Phase	Number
Grape-derived EVs	Colon tumor	Curcumin	I	NCT01294072
Ginger-derived EVs	Irritable bowel disease	Curcumin	Pilot clinical trial	NCT04879810
Grape-derived EVs	Head and neck cancer oral mucositis	/	I	NCT01668849
<i>Aloe</i> and ginger-derived EVs	Polycystic ovary syndrome	/	Preliminary clinical trial	NCT03493984

8. Summary and prospects

The use of PDEVs for drug transport is gaining widespread attention. Their good biocompatibility, nanoscale particle size, and unique anti-tumor potential provide a new platform for tumor-targeted therapy, enhanced immunotherapy, and the realization of combination immunotherapy⁴². Some EVs derived from Chinese herbs contain the active substances of Chinese herbs and exhibit good anti-tumor value^{42,122}. For instance, by preventing tumor cell proliferation, upregulating proteins associated with apoptosis, and downregulating proteins linked to anti-apoptosis, EVs derived from garlic, ginseng, ginger, and *Artemisia annua* may inhibit the development of tumor cells^{16,337}. Furthermore, PDEVs may improve the anti-tumor immune response by modifying the immunosuppressive microenvironment¹⁶⁰. Toll-like receptor-related signaling pathways may be triggered by ginseng-derived EVs, which can encourage anti-tumor macrophage polarization⁴⁰. *Artemisetic*-derived EVs can activate the intracellular cGAS–STING pathway of macrophages to upregulate the anti-tumor response¹⁶⁰.

Because PDEVs have more sources, a more straightforward manufacturing method, and more effortless drug loading, they may be employed as an effective and safe drug delivery carrier in comparison to traditional nanocarriers and MDVs^{5,311}. In addition, PDEVs can effectively cross biological barriers; for example, ginsenoside Rg3 in ginseng vesicles can bind with GLUT1 of cerebrovascular endothelial cells to mediate ginseng-derived EVs to cross the blood-brain barrier and achieve brain tumor targeting³⁹. The construction of anti-tumor drug delivery systems based on PDEVs has been widely used, such as the delivery of small interfering RNA and chemotherapy drugs^{5,323}.

Additionally, PDEVs can be modified and engineered through physical, chemical, and genetic means to enhance their drug loading-ability, targeted delivery, and anti-tumor effects. The engineered PDEVs can better target tumor sites and achieve precise drug delivery timing. By boosting the body's anti-tumor immune response, cancer immunotherapy, a novel treatment approach, may eradicate tumors and have great application potential. However, its therapeutic use is limited due to the tumor microenvironment's complicated composition, high metabolic level, and sparse immune cell population. Immunotherapy may be enhanced by combining it with photothermal or photodynamic chemotherapy. This combination can lessen adverse side effects and provide synergistic tumor growth suppression. Engineering PDEVs may achieve a combination treatment to co-deliver immunomodulators and other therapies, as they serve as a delivery vector that can regulate the immune milieu and suppress tumors. Furthermore, immunomodulatory PDEVs may increase the effectiveness of immunotherapy by acting as drug transporters. Thus, altered PDEVs have great potential and may be used as a novel platform for combination immunotherapy.

Through physicochemical modification, genetic modification, and membrane hybridization, engineered PDEVs can accurately target the tumor microenvironment, integrate multiple treatment modalities, overcome delivery bottlenecks such as toxicology and drug resistance in traditional combination therapy, and even achieve sequential delivery *in vivo* and personalized treatment^{338,339}. The membrane fusion engineering strategy can enable PDEVs to obtain the advantages of two or more membranes and construct new delivery carriers based on PDEVs^{166,340}. Therefore, the membrane fusion process of PDEVs and liposomes or EVs from tumor cells is feasible for improving drug loading, long-cycle

stability, and targeting and can be a focus of future research. In addition, EV-based personalized nano-vaccines have shown promising application prospects in oncology and infectious diseases⁸. A batch of EV-based nano-vaccine delivery systems is under clinical evaluation and has shown promising results⁸. PDEVs possess excellent safety, lymphatic accumulation capacity, antigen presentation ability, easy modification characteristics, good stability, and a wide range of sources³⁴¹. Consequently, we believe that PDEV-based nano-vaccines may be a crucial avenue for future vaccine research and development, which is essential for the prevention and treatment of cancer in humans^{342–345}.

Although the research and application of PDEVs have made significant progress, several critical challenges remain. First, there aren't enough effective ways to produce PDEVs on a large scale and store them steadily. Typically, the extracted PDEVs are stored at -80°C , and the traditional extraction and separation methods are more complicated and time-consuming. Second, to enhance the therapeutic efficacy and drug delivery efficiency of PDEVs, novel drug delivery techniques must be developed. Third, the *in vivo* process of PDEVs needs more in-depth research. Studies on the absorption, distribution, metabolism, and excretion of PDEVs are relatively limited, and new methods can be developed to track the specific *in vivo* reactions of PDEVs in the future. Fourth, the potential reaction between PDEVs and cargo. When PDEVs serve as a delivery carrier for immunotherapy combination therapy, the potential reaction between PDEVs and co-loaded drugs must be thoroughly studied. Finally, the effect of the engineering treatment of PDEVs on its active ingredients needs to be further explored to ensure that the engineering treatment of PDEVs does not alter its original properties.

9. Conclusions

This paper reviewed the basic characteristics of PDEVs, including the formation mechanism and the main pathway of PDEV uptake by receptors, the main components of PDEVs, the anti-tumor mechanism, drug delivery methods, and drug delivery applications as delivery carriers. It also discussed the current research status of engineering strategies, combined immunotherapy, and clinical research. Overall, engineered PDEVs are promising as delivery vectors for cancer therapy and to enable combined immunotherapy.

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Drawing picture. Jiaxin Guo, Jiale Li, Wenjing Chen: Validation. Kuanhan Feng: Investigation. Liang Feng, Wen Zhang: Investigation and Validation.

Conflicts of interest

The authors have no conflicts of interest to declare.

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